State of California
The Resources Agency
Department of Water Resources
Division of Planning and Local Assistance

California State Water Project

Coordinated Pathogen Monitoring Program

Draft Project Report

May 1999

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Governor	Secretary for Resources	Director
State of California	The Resources Agency	Department of Water Resources

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EXECUTIVE SUMMARY

Recommendations were made in the *California State Water Project Sanitary Survey Update Report 1996* to address the potential threat to human health of microbial contaminants in State Water Project waters, including *Giardia lamblia* and *Cryptosporidium parvum*. It was recommended that the microbiological safety of SWP source waters be comprehensively evaluated, and monitoring be coordinated with municipal SWP contractors to make data collected by the contracting agencies comparable to data collected from within the SWP system and its source waters by the California State Water Project Coordinated Pathogen Monitoring Project.

In addition to the recommendations made in the sanitary survey update report, the U.S. Environmental Protection Agency's Information Collection Rule was promulgated in 1996, with the actual ICR Study beginning in July 1997. This project was designed to augment data which was collected by the microbiological monitoring required by the ICR, which also includes both *Giardia* and *Cryptosporidium*. Project oversight and review was provided by the Sanitary Survey Action Committee.

The project design incorporated both monthly samples and storm event samples. Sampling was conducted for the 12-month period of November 1996 through October 1997 by the Department of Water Resources at source water locations and at locations within the SWP, and by Metropolitan Water District of Southern California at Castaic and Silverwood lakes. Flood event sampling was added to the storm event monitoring as a result of the January 1997 floods. MWD also provided an initial workshop for all agencies participating in the study to ensure sampling consistency, along with technical support as needed throughout the study.

Analytical Methods

USEPA's ICR methods for *Giardia* and *Cryptosporidium*, and for *Clostridium perfringins* were used for this study. A Method Detection Limit goal of 10 cysts or oocysts/100 L (total immunofluorescence antibody [IFA] count) for the protozoa was specified for this project. Total, fecal coliforms, and *E. coli* were analyzed using the 5-tube, 5-dilution most probable number method from *Standard Methods for the Examination of Water and Wastewater,* 19th Edition.

The USEPA ICR Protozoan Method has several performance characteristics which should be considered when interpreting results. It is widely recognized as being tedious, time consuming and difficult to run, with poor recovery, precision, and accuracy, characteristics which were evident in this study. Experience has demonstrated that the ICR method underestimates both protozoan concentration and frequency of detection. The method is not intended to determine either the viability or infectivity of cysts or oocysts.

Split Spiked Matrix Recovery Study

Quality assurance and quality control were provided as required by the analytical methods, in compliance with the ICR where applicable, and in accordance with existing Department of Water Resources' Division of Planning and Local Assistance Quality Assurance/Quality Control protocols. In addition, a split spiked matrix study was conducted using matrix water obtained from five locations distributed throughout the project area to determine the recovery of the method in the various water matrices found throughout the project area.

The average recoveries of spiked *Giardia* cysts (2.53 percent) and *Cryptosporidium* oocysts (0.35 percent) were very low, the standard deviations were

large, and 50 percent of the *Cryptosporidium* spiked samples were non-detects. The results of this recovery study are indicative of the performance concerns related to the use of this method, and of the difficulties in interpreting the results obtained using it. Actual protozoan concentrations and detection frequency are likely much higher than the results indicate. The split matrix spike results did, however, demonstrate that method performance is generally consistent with all water matrices obtained from within the project area.

Results

All *Giardia* and *Cryptosporidium* results discussed in this report are reported as Total IFA counts, which represents a conservative use of the data. Throughout the study, *Cryptosporidium* detection frequencies were low compared with those of *Giardia*. For stations where both *Giardia* and *Cryptosporidium* were detected, the percent of samples positive for *Giardia* was generally much higher than for *Cryptosporidium*. While *Cryptosporidium* concentrations may actually be lower than those of *Giardia*, the recovery of *Cryptosporidium* was approximately 10 times lower than *Giardia* with this in this study. The poor recovery of both *Giardia* and *Cryptosporidium* using the ICR Protozoan Method and identified in the Split Matrix Spike Recovery Study discussed above must be considered when interpreting the results of this study. Actual numbers of *Cryptosporidium* and *Giardia* at all sites may be significantly higher based on the poor recoveries of this method.

Both *Giardia* and *Cryptosporidium* detection was much more frequent in samples from the Sacramento River, San Joaquin River, and other source waters of the SWP compared with samples from locations within the SWP system itself, which includes the aqueduct and reservoirs. The majority of results from samples collected from sampling locations either in the California Aqueduct or in the SWP reservoirs were below the detection limit for both protozoa.

Giardia was detected in 50 percent of the source water samples and in only 2 percent of the SWP samples. *Cryptosporidium* was present in 11 percent of the source water samples and in 5 percent of the SWP samples. Fate processes capable of influencing cyst and oocyst concentrations are not well defined for these organisms.

The low recovery of *Giardia* and *Cryptosporidium* obtained using the ICR Protozoan Method resulted in effective detection limits much higher than the study goal of 10 cysts or oocysts per 100 L. The practical result of this method performance is that sampling locations with either no protozoa detected, or a low frequency of detection cannot be considered to be free of protozoa. Protozoa could still be present at significant levels, but lower than the method was capable of detecting and quantifying.

The detection frequency, geometric mean, and range of positive results for both protozoans were greater in the storm and flood event samples collected in the wet season compared with the samples collected on a monthly basis, which includes both wet and dry season results. Both protozoa were detected more frequently in the wet season samples relative to the dry season samples; *Giardia* wet season/dry season percent positive samples was 31 percent/16 percent, and *Cryptosporidium* was 11 percent/2 percent.

The range of positive *Clostridium perfringins* concentrations in monthly samples was 2 - 800 CFUs/100 mL, with a geometric mean of 46.9 CFUs/100 mL. As with Giardia and Cryptosporidium, detection frequency was higher in storm and flood event samples compared to monthly samples, and higher in river and Delta source waters relative to water in the SWP system. The highest frequency of detection was at the North Bay Aqueduct intake at Barker Slough, which also had the highest geometric mean of all monthly sampling locations for *C. perfringens*.

As with the protozoans and *C. perfringens*, total/fecal coliforms and *E. coli* detection frequencies and concentrations were highest in the Sacramento River, San Joaquin River, and in the Delta compared with the SWP aqueduct and reservoirs. Storm and flood event sample detection frequency and geometric means were also higher than those of the monthly samples for these organisms.

Additional samples were collected during the January 1997 floods in order to gain information about the pathogen levels of flood waters. Selected storm event sampling locations were sampled during the week of January 6-10, 1997, with several additional locations added to sample flood waters in specific areas.

The flood samples as a group had the highest geometric mean for *Cryptosporidium*, total/fecal coliforms, *E. coli* , and *C. perfringens* when compared with either the monthly or event sample group results for all organisms. Detection frequency, as percent of positive samples, was higher for all organisms/organism classes in the flood event group than for either the monthly or event sample groups. The flood group *Giardia* detection frequency was 70 percent positive samples, with *Cryptosporidium* at 40 percent positive.

Correlation Analysis

Correlation analyses were conducted to determine if the organism and organism classes or turbidity were correlated with each other. The results of the correlation analysis indicate that the data are not well correlated. Only the relationship between fecal coliforms and *E. coli* exhibits a correlation coefficient greater than 80 percent and only two more sets (fecal vs. *C. perfringens* and *C. perfringens* vs. *Giardia*) exhibit coefficients greater than 50 percent. Correlation analyses were also run for those individual sites where adequate sample data existed. The results indicate that any correlation between these parameters was likely to be site specific, and may also be

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seasonally specific or episodic. This finding was consistent with other efforts to find surrogates for *Giardia* and *Cryptosporidium*.

CONCLUSIONS

Analytical

- The USEPA ICR Protozoan Method demonstrated poor recovery, accuracy, and precision in the CPMP Study. The detection frequency and concentrations of both protozoa were likely higher than the analytical results indicate.

- Due in part to the low protozoan recovery, the detection limit calculated for an ICR protozoan analytical result does not reflect the actual detect limit, which was most likely higher. If a detection limit goal of 10 cysts/oocysts per 100 liters had not been set, even fewer detections would have been observed.

Results

- The range, geometric mean, and percent positive samples of the CPMP event samples were higher compared with the monthly samples. Storm and flood waters contained higher concentrations of protozoa more frequently than "average" waters sampled on a monthly basis.

- When wet season sample results were compared with dry season results, both protozoa were detected more frequently and at higher concentrations in the wet season compared to the dry season. *Giardia* was detected more frequently than *Cryptosporidium*.

- Giardia, *Cryptosporidium*, *C. perfringens*, and total/fecal coliforms and *E. coli* detection frequency and concentrations were highest in the Sacramento River, San Joaquin River, and Delta source waters compared with the SWP aqueduct and reservoirs. This difference did not appear to be related to any change in the performance of the USEPA ICR Protozoan Method caused by possible physical or chemical changes in the water as it moves from the source through the SWP system, a distance of nearly 600 miles. These protozoans may still be present in significant numbers in the SWP based on the poor recovery of the method.

- *Cryptosporidium* was detected less frequently and at lower concentrations compared to *Giardia* in the CPMP Study. While *Giardia* may have actually been present more often and at higher concentrations than *Cryptosporidium*, the recovery of *Cryptosporidium* by the analytical method was approximately 10 times less than *Giardia* in this study.

- The flood samples as a group had the highest geometric mean for *Cryptosporidium*, total/fecal coliforms, *E. coli* , and *C. perfringens* when compared with either the monthly or event sample group results for all organisms. Detection frequency, as percent of positive samples, was higher for all organisms/organism classes in the flood event group than for either the monthly or event sample group.

Correlation Analyses

- The results of the correlation analysis indicate that the data were not well correlated. Only the relationship between fecal coliforms and *E. coli* exhibited a correlation coefficient greater than 80 percent.

- Correlation between the organisms, organism classes, and turbidity are likely to be site specific, and may also be seasonally specific or episodic.

- Correlation may also be affected by method performance, i.e., the poor precision and accuracy observed for the protozoa in this study may preclude a quantitative correlation from being determined, should one present.

- The lack of correlation between organisms may be due to different ecological characteristics of the species. Surrogates may not be suitable predictors for the occurrence of protozoa.

General

- Experience has demonstrated that both protozoan concentrations and frequency of detection are underestimated by the ICR Protozoan Method. An improved analytical method is needed for analysis of *Giardia* and *Cryptosporidium* in raw and finished waters. The current ICR Protozoan Method exhibited poor recovery, accuracy, and precision for both protozoans in this and other studies. The method was inadequate based on the high cost and effort required to obtain results, along with the resulting performance-related qualitative and quantitative limitations placed on the interpretation and use of the data experienced in this study.

Chapter 1

INTRODUCTION

The *California State Water Project Sanitary Survey Update Report 1996* (DWR 1996) made recommendations to address the potential threat to human health of microbial contaminants in SWP waters, such as *Giardia lamblia* and *Cryptosporidium*. These recommendations included:

1. Sampling for *Giardia lamblia* and *Cryptosporidium* should be added, and total and fecal coliform sampling should be carried out.

2. Further investigation of each watershed should be conducted to further evaluate the potential sources of microbial contaminants identified.

3. The microbiological safety of SWP source waters should be comprehensively evaluated on an ongoing basis, and should include implementation of the following elements:

    a. Institute total and fecal coliform and monitoring of SWP source water at key locations.

b. Work with municipal SWP contractors to coordinate monitoring in such a manner as to make data collected by the contracting agencies comparable to data collected from within the SWP system.

c. Monitoring data from contracting agencies should be accumulated on an ongoing basis, along with data collected from within SWP.

d. Results of the data analyses and evaluations should be shared on an ongoing basis among municipal contractors and DWR staff.

In addition to the recommendations made in the sanitary survey update report, the USEPA Information Collection Rule was promulgated in 1996, with the actual ICR Study beginning in July 1997. The rule required large public water systems (systems serving a population of ≥100,000 persons) to routinely monitor influent water for several chemical and microbiological constituents, which include total and fecal coliforms, *Giardia lamblia*, *Cryptosporidium*, and viruses monthly for 18 months. The rule also required these large public water suppliers to routinely monitor finished water if, during any of the first 12 months of monitoring of the treatment plant influent, the following were detected:

1. 1,000 or more *Giardia lamblia* cysts/100 L,
2. 1,000 or more *Cryptosporidium* oocysts/100 L; or
3. One or more total culturable viruses/L.

This project was developed based on recommendations made in the sanitary survey update report and to augment data which was collected through the microbiological monitoring required by the ICR. The data from this monitoring program, combined with the ICR monitoring data obtained by public water suppliers (using the SWP as a source of drinking water), provides a substantial set of microbiological data

which may be used to evaluate and assess the baseline microbiological status of SWP source waters used for drinking water.

Project oversight and review was provided by the SSAC. This Committee included staff from the State Water Contractors organization, individual state water contractors, DWR's DPLA and O&M, MWD, USEPA Region IX, Department of Health Services, CALFED, and the State Water Resources Control Board. The study was coordinated and managed by the Municipal Water Quality Investigations Program of DWR's DPLA.

GIARDIA AND *CRYPTOSPORIDIUM*

The single celled protozoans *Giardia lamblia* and *Cryptosporidium parvum* are commonly found in surface waters, and in some cases groundwaters, throughout the U.S. They are intestinal parasites in both humans and animals, often causing diarrhea and other gastrointestinal/gastroenteritis signs and symptoms. Transmission by water and food is most common, although other fecal-oral routes are possible. Both organisms have a stable dormant stage which can persist for some time in both terrestrial and aquatic environments, and cause infection and active disease upon ingestion by suitable hosts, which can include humans.

Both organisms have been implicated in outbreaks of disease with drinking water as the mode of transmission. There have been several outbreaks of cryptosporidiosis in the U.S. The majority of individual cases were found to be related to drinking water derived from surface water sources. Sources of *Cryptosporidium* attributed to these outbreaks include wastewater discharges and agricultural runoff (Solo-Gabriele and Neumeister 1996; Juranek and others 1995; Roefer and others 1996). *Cryptosporidium* is particularly resistant to the chemical disinfectants, such as chlorine, which are used to treat drinking water (MMWR 1995).

While generally self-limiting diseases in healthy individuals, they may be more dangerous to persons who are immunocompromised, which includes those with HIV-infections or AIDS, with genetically determined immune system deficiencies, those taking immunosupressive drugs related to organ transplants, cancer chemotherapy patients, and the very old and the very young, (Butler and Mayfield 1996; FDA 1992). *Cryptosporidium* is of particular concern, since unlike *Giardia*, there is currently no effective antibiotic available to treat an infection in these individuals.

SCOPE

CPMP was intended to link and augment the current and proposed monitoring programs of MWD, DWR's O&M and DPLA's MWQI programs, and the USEPA's ICR Monitoring Study. The project design incorporated both monthly and storm event samples, with monthly sampling started in November 1996 and continued through April 1998. Sampling of flood waters from the January 1997 floods was added to the program at both existing sampling locations and at four additional locations.

Sampling locations were selected to include the source waters of the SWP, the Delta, the SWP's California Aqueduct, and the major reservoirs comprising the SWP system (Appendix F). The sampling locations included the Sacramento River above and below the American River, the Sacramento River above and below the City and County of Sacramento's principal publicly owned treatment works outfall, the San Joaquin River above and below the City of Stockton's publicly owned treatment works outfall, the Delta, the SWP's California Aqueduct, and SWP reservoirs. The sampling locations are further described in Appendix G.

USEPA's ICR methods for both *Giardia/Cryptosporidium* and *C. perfringins* were used for this study. This allows comparison with the results obtained by utilities using SWP water and required to participate in the ICR Study using these protozoan

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methods. Total, fecal coliforms, and *E. coli* samples were collected and analyzed using the 5-tube, 5-dilution MPN method (APHA 1995).

Sampling was conducted by DWR's DPLA at source water locations, by DWR's O&M at locations within the SWP, and by MWD at Castaic and Silverwood lakes. Kern County Water Agency assisted with sampling at the Check 29 sampling location. MWD provided an initial workshop for all sampling agencies to ensure sampling consistency, along with technical support as needed throughout the study.

Sampling was conducted over an 18-month period in two phases. After the first 12 months of sampling, the number of sampling stations was reduced. Sampling for the last 6 months of the study was concentrated on those locations having the greatest detection frequency, which included the more northern SWP stations, the Sacramento River, the San Joaquin River, and Delta sampling locations.

Monthly Monitoring Locations

During the first 12 months of sampling, monthly samples were collected at locations listed in Table 1-1 and displayed in Figure 1-1. Sampling sites in the Delta and its tributaries are shown in greater detail in Figure 1-2. MWD conducted monthly sampling at Castaic and Silverwood lakes from the intakes for the Jensen and Mills Water Treatment Plants, respectively, and at Devil Canyon. The source water for these plants at the time of sampling consisted of 100 percent SWP water.

Table 1-1.

Monthly Monitoring

Sampling Site	Sampled by:
Sacramento River at Bryte Bend, at the marina	DPLA
Sacramento River above Sacramento Regional Wastewater Treatment Plant and below confluence with American River at the Miller Park dock	DPLA
Sacramento River below Sacramento Regional Wastewater Treatment Plant at Greenes Landing	DPLA
San Joaquin River at Vernalis at the Airport Road bridge	DPLA
Stockton Wastewater Treatment Plant[1] at Holt Road	DPLA
Banks Pumping Plant[2] at Bethany Reservoir	O&M
Delta-Mendota Canal at McCabe Road	O&M
Arroyo Valle Creek Inflow to Lake Del Valle (when flowing, approximately 5 months/year), at the creek mouth	O&M
California Aqueduct at Check 29	KCWA/O&M
Pyramid Lake at the tower in Elderberry Forebay, release from Elderberry Forebay to Castaic	O&M
Castaic Lake influent to Jensen Water Treatment Plant	MWD
Silverwood Lake, influent at Mills Water Treatment Plant or Devil's Canyon	MWD
Lake Perris at the outlet tower	O&M
Barker Slough Pumping Plant, North Bay Aqueduct Intake	O&M

[1] Samples are taken downstream of the Stockton POTW outfall at or shortly after the midpoint of an ebb tide at the sampling site to ensure flow is toward the Delta.

[2] Sample collected at the inlet to Bethany Reservoir just downstream from Banks Pumping Plant.

Figure 1 - 1
Coordinated Pathogen Monitoring Program for the State Water Project

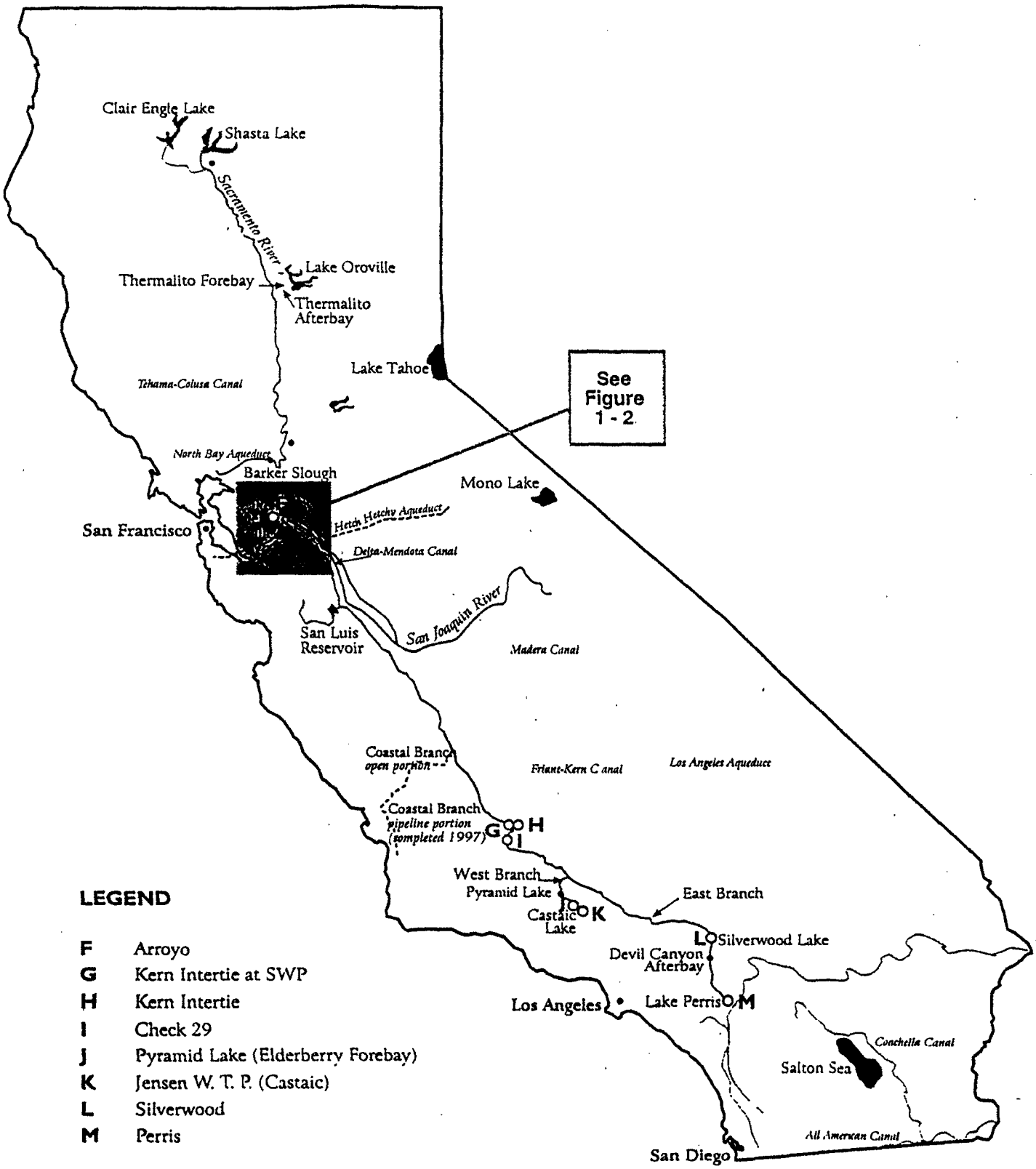
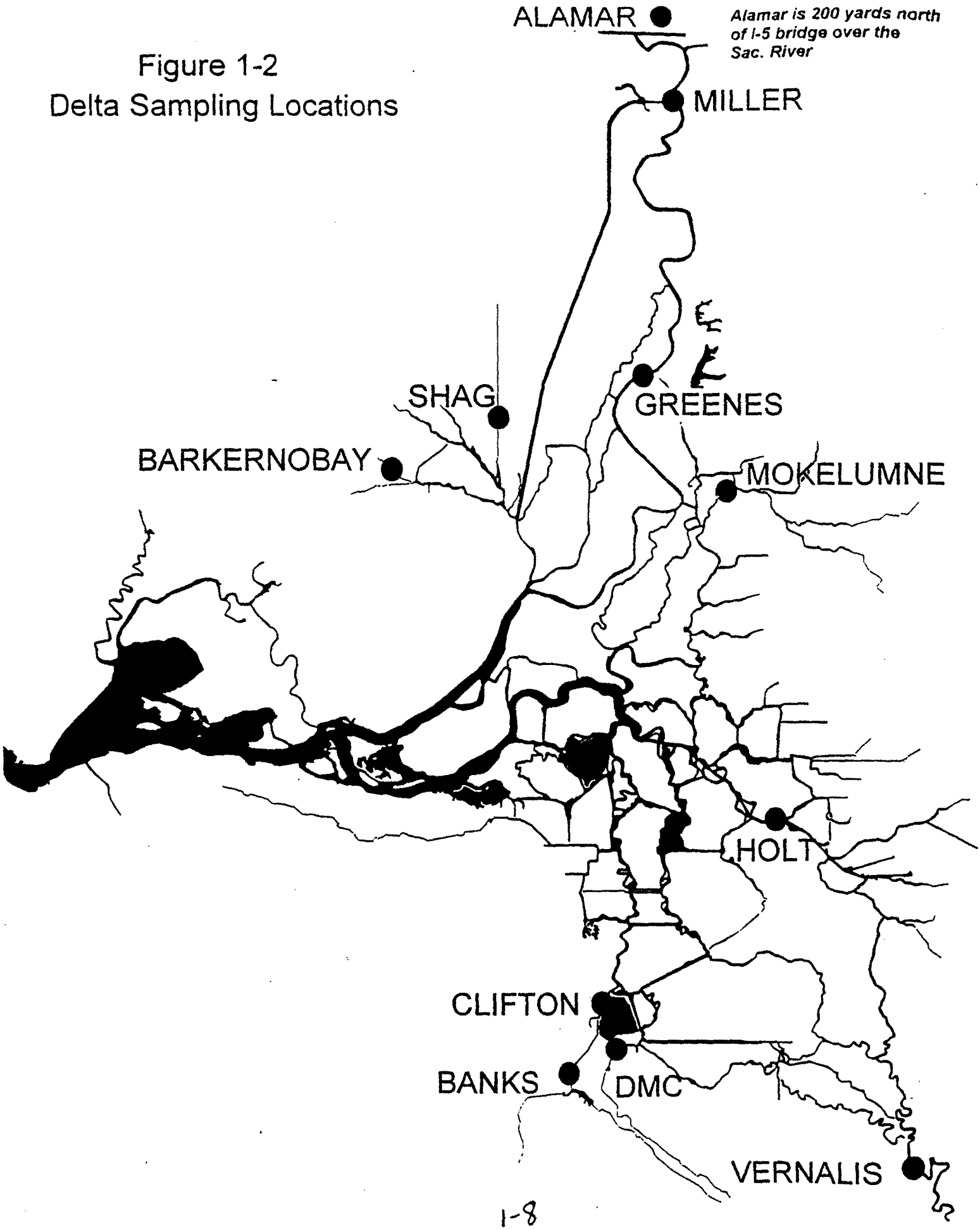


Figure 1-2
Delta Sampling Locations



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Event Monitoring Locations

Storm and flood event sampling locations were included in the study. As with the monthly samples, the number of sampling locations was reduced after 12 months of sampling. Storm event samples were obtained during the first major storm of the wet season and during two additional major storm events. The storm and flood event sampling locations are shown in Figures 1-1 and 1-2.

Four flood-related locations were added to the 12 storm event monitoring locations as a result of the January 1997 floods. Flood event samples were collected during January 6-10, 1997 at the 12-storm event sampling locations, with two sites added to monitor the flood waters of the Mokelumne River and the Yolo Bypass. Two sampling locations were added coinciding with the opening of the Kern River Intertie to the California Aqueduct during the period of flooding. One sample was collected from the Kern River prior to its confluence with the California Aqueduct and one sample was collected from the California Aqueduct upstream of this confluence with the Kern River at the intertie.

Storm Event Monitoring Criteria

A storm event for the purpose of this study was defined as rainfall of sufficient intensity and duration resulting in measurable surface runoff, or a measurable change in existing runoff, from interior areas of the watershed into the system of streams, creeks, rivers, or other channels comprising the drainage system of the watershed. There are various factors related to the nature of the storm and specific to the watershed that can influence surface runoff.

Each watershed in this project was expected to respond differently to rainfall events. A general guideline of 1 inch of rain in a 24 hour period was used as a trigger

to assess a storm event for monitoring purposes. Whenever possible, river stage and gauging information was also used to determine the rising arm of the storm event hydrograph, particularly on the Sacramento and San Joaquin rivers.

Ideally, a gauging station or flow meter measuring either the depth or the velocity of water in the stream would be located above the sampling site to determine the hydrograph of the storm event runoff. It was important for the purposes of this study that storm events be sampled on the rising side of the storm hydrograph, but before the crest or time of greatest flow or depth of water in the stream is reached. An upstream gauging station or flow meter could calculate or predict the rising hydrograph in order to determine the optimum time of sampling. After the sample has been taken, this type of data can also be used to retroactively determine the point on the hydrograph when the sample was obtained. Any tidal influences or regulated flows would have to be considered. Selected sites for the CPMP event-based monitoring are shown in Table 1-2.

Since it was unlikely that gauging stations or flow meters were present, and/or would be placed in the channel at all sampling sites prior to the storm event, best professional judgement and a familiarity and knowledge of the watershed and how it responds to storm events was employed by the sampler to estimate the appropriate point on the hydrograph to collect the storm-event sample. When storm-event samples were collected during the week when a monthly sample was scheduled to be collected, the monthly sample was not collected.

Table 1-2.

Event-Based Monitoring

Sampling Site	Sampled by:
Sacramento River at Bryte Bend, at the marina	DPLA
Sacramento River above Sacramento Regional Wastewater Treatment Plant and below confluence with American River, at Miller Park dock	DPLA
San Joaquin River at Vernalis, at the Airport Road bridge	DPLA
Banks Pumping Plant at Bethany Reservoir	O&M
Clifton Court at the West Canal intake near radial gates	O&M
Delta-Mendota Canal at McCabe Road	O&M
Arroyo Valle Creek Inflow to Lake Del Valle, near the creek mouth	O&M
California Aqueduct, Check 29 [1]	KCWA/O&M
Pyramid Lake at the Piru Creek gauging station	O&M
Castaic Lake at Elderberry Forebay [2]	O&M
Silverwood Lake [3]	O&M
Barker Slough Pumping Plant	O&M
Mokelumne River at New Hope [4]	O&M
Shag Slough at Liberty Island Bridge [4]	DPLA
Kern River Intertie just prior to confluence with the California Aqueduct[4]	O&M
California Aqueduct at MI 241.02 just upstream of the Kern River Intertie[4]	O&M

[1] Inflow to the San Luis Reach of the California Aqueduct from Cantua and Salt Creeks may be used as a storm event monitoring trigger for this site.

[2] a. Fish Creek and Castaic Creek confluence at the lowest debris basin above Elderberry Forebay
b. Fish Creek - if no water in debris basin
c. Castaic Creek
d. Elizabeth arm of lake at the gauging station

[3] a. Miller Canyon gauging station
b. Cleghorn drainage
c. Sawpit

[4] Flood event related sites.

Chapter 2

METHODS, QUALITY ASSURANCE, AND QUALITY CONTROL

METHODS

All samples obtained for this monitoring program were analyzed for the following microorganisms by the indicated analytical methods, unless exceptions are noted:

1. *Giardia* and *Cryptosporidium*
   a. Analysis: USEPA ICR Protozoan Method For Detecting *Giardia* Cysts and *Cryptosporidium* Oocysts in Water by a Fluorescent Antibody Procedure, Section VII, EPA/600/R-95/178, April 1996 (USEPA 1996a).
   b. Sampling: Information Collection Requirements Rule - Protozoa and Enteric Virus Sample Collection Procedures, EPA/814-B-95-001, June 1995 (USEPA 1996b).
   c. A 100-liter volume of water was filtered if at all possible. If turbidity was greater than 160 NTU, a 4-liter grab sample was submitted for analysis in place of the filtered sample. The option for collection of a grab sample was a project specific change to the ICR sampling protocol to allow for sampling of highly turbid waters.
   d. A MDL goal of 10 cysts or oocysts/100 L (total IFA count) was specified for this project. A maximum of five slides were analyzed and the results of each slide combined to achieve this detection limit. The results of each individual slide were also reported separately for each sample analyzed.

2. Total and fecal coliforms, and *E. coli*

   a. Standard Methods for the Examination of Water and Wastewater, 19th Edition, (APHA 1995). 5 Tube - 5 Dilution Standard Total Coliform/Fecal Coliform Fermentation Technique, with *E. coli* Procedure added. Sections referenced include: Section 9221 A-C and Section 9221 F.

   b. A 100 mL grab sample was collected in sterile containers.

3. *C. perfringens*

   a. USEPA ICR Membrane Filter Method for *C. perfringens*, Section XI, (EPA/600/R-95/178), April 1996 (USEPA 1996e).

   b. A 100 mL grab sample was collected in sterile containers.

Analytical Laboratory

Samples collected by MWD, DWR's O&M and DPLA, and KCWA for *Giardia* and *Cryptosporidium,* total and fecal coliforms/*E. coli*, and *C. perfringens* were sent to BioVir Laboratories (Benicia, California) for analysis.

Sample Holding Time

The holding times established for this study were as follows:

1. *Giardia* and *Cryptosporidium*: 96 hours
2. Total and fecal coliforms, and *E. coli*: 24 hours
3. *C. perfringens*: 24 hours

Samples were collected, packaged, and shipped as soon as possible to meet these holding times. When collecting samples, the *Giardia/Cryptosporidium* sample

was collected first, since this sample requires more time to collect. The samples collected for total and fecal coliforms, *E. coli*, and *C. perfringens* were collected last and just prior to leaving the sampling site in order to conserve sample holding time.

Sampling Schedule

Storm-event sampling began with the first storm of the winter wet season of 1996-1997, which occurred in late October 1996, and was repeated the following year. Monthly samples were collected beginning in November 1996 and ending in April 1998.

QUALITY ASSURANCE AND QUALITY CONTROL

QA/QC was provided as required by the analytical methods, in compliance with the ICR where applicable, and in accordance with existing DWR DPLA QA/QC protocols. In addition, split matrix spike samples were collected from sampling locations throughout the project area and analyzed by BioVir Laboratories.

1. Analytical precision: Detection limits improve with the reading of more slides, and reporting results based on all slides taken together. Reading more than one slide would be expected to give some indication of precision between slides. When reading more than one slide to achieve the detection limit, BioVir Laboratories reports the results of each slide separately, with the results combined for all slides for detection limit purposes (total IFA count), which is reported in this study.

2. The USEPA ICR Performance Evaluation sample analysis for *Giardia/Cryptosporidium* was completed prior to the start of the ICR Study. In combination with a laboratory facility evaluation, these performance evaluations were designed to determine which laboratories would be approved to participate

in the ICR Study, which began in July 1997. BioVir was approved to participate in the study and analyze ICR samples (see Appendix A).

Laboratories had to meet specific QC and PE study requirements during the course of the ICR 18-month study to maintain USEPA approval to continue to participate. Utilities participating in the study were required to use an USEPA ICR-approved laboratory. In addition to attaining initial EPA approval to participate in the ICR Study, BioVir maintained it's ICR approval through continued acceptable performance on monthly performance evaluation (PE) samples since the ICR Study began.

3. Results of the weekly IFA positive and negative batch samples required by the ICR Protozoan Method are reported along with the data. Also required by the ICR Protozoan Method, are monthly data on the recovery of cysts and oocysts from spiked QC samples (Appendix B).

4. BioVir is subject to quarterly California Department of Health Services certification for microbiological testing (coliforms and *E. coli*). Laboratories must maintain State certification under the Drinking Water Certification Program to participate in the ICR Study.

5. The results of the total/fecal coliforms, *C. perfringins*, and *E. coli* quality control results are in Appendix B.

COLIFORMS

Analyses for coliforms were performed by BioVir Laboratories in accordance with 9221B and 9221F of *Standard Methods for the Examination of Water and Wastewater, 19th edition* (APHA 1995).

Appendix B-1 shows the results of internal laboratory quality control samples and parameters pertaining to analyses for coliforms. This table shows the quality control results of exceedances of media expiration or hold time limits; media pH criteria; media sterility; media growth controls for lauryl tryptose broth (LTB) during the presumptive phase, brilliant green lactose bile (BGLB) broth during the confirmed phase, and *E. coli* with 4-methylumbelliferyl-β-D-glucuronide (EC-MUG) broth during the completed phase; incubation temperature checks at 35°C; water bath temperature checks at 44.5°C; and exceedances of hold time limits.

As shown in Appendix B-1, with the exception of holding times, all quality control acceptance criteria for the analyses of coliforms were met. Prior to implementation of this study, an extended hold time limit of 24 hours was established to accommodate the longer transport time required for samples collected at southern California sampling sites. According to 9060B of Standard Methods, the usual hold time limit (from time of sampling to time of sample processing) is six hours with an elapsed time between collection and examination of no more than 24 hours recommended. Of the 169 samples reviewed and listed in Appendix B-1, only 89 samples (53 percent) included results on holding times. Of these 89 samples, the holding times for 7 samples (8 percent) exceeded the established 24 hour hold time limit (Table 2-1):

Table 2-1.

Coliform Samples With Hold Time Limits Exceeded

Sample Number	Analytical Results (MPN/100mL)		
	Total Coliforms	Fecal Coliforms	*Escherichia coli*
C970194	170	17	17
S12107	<2	<2	<2
S12109	No Results		
S12133	<2	<2	<2
S12134	<2	<2	<2
L20887-3	No Results		
SJ-1518	No Results		

Of the 7 samples in which the 24 hour hold time limit was exceeded, three samples (S12107, S12133, and S12134) had results of less than detection limit and three samples (S12109, L20887-3, and SJ-1518) were not analyzed. For these six samples, the hold time limit exceedances were of no significance. For sample number C970194, the analytical results for total coliforms (170 MPN/100 mL), fecal coliforms (17 MPN/100 mL), and *E. coli* (17 MPN/100 mL) were compared with the other results from the same sampling site (HOLT). At this sampling site, the range of results for total coliforms was 50 - 5,000 MPN/100 mL; for fecal coliforms, the range of results was 11 - 1,700 MPN/100 mL; and for *E. coli*, the range of results was <2 - 1,700 MPN/100 mL. The analytical results for sample number C970194 were consistent with the majority of the results for that sampling site, which were at the lower end of the ranges. Based on this comparison, it would seem unlikely that the hold time limit exceedance was significant.

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C. PERFRINGINS

Analysis for *C. perfringens* spores was performed by BioVir Laboratories in accordance with the *ICR Microbial Laboratory Manual*, EPA/600/R-95/178, April 1996.

Appendix B-2 shows the results of internal laboratory quality control samples and parameters pertaining to analyses for *C. perfringens* spores. This table shows the quality control results of exceedances of media expiration or hold time limits; media pH criteria; media sterility; membrane filter controls; media growth controls for modified mCP agar during the presumptive phase; media growth controls for modified iron milk medium during the confirmed phase; and exceedances of hold time limits.

As shown in Appendix B-2, with the exception of holding times, all quality control acceptance criteria for the analyses of *C. perfringens* spores were met for the samples reviewed. Although *C. perfringens* spores can survive for extended periods at 1-4°C, a hold time limit of 24 hours was established to maintain consistency with the hold time limit established for coliforms. Of the 138 samples listed in Appendix B-2, only 91 samples (66 percent) included results on holding times. Of these 91 samples, the holding times for 6 samples (7 percent) exceeded the established 24 hour hold time limit (Table 2-2).

Of the 6 samples in which the 24 hour hold time limit was exceeded, four samples (C970194, S12107, S12133, and S12134) had results of less than detection limit and two samples (S12109 and SJ-1517) were not analyzed. For these six samples, the hold time limit exceedances were of no significance.

Table 2-2.

***C. perfringens* Samples With Hold Time Limits Exceeded**

Sample Number	*C. perfringens* (CFU/100mL)
C970194	<100
S12107	<5
S12109	No Results
S12133	<10
S12134	<10
SJ-1517	No Results

GIARDIA AND CRYPTOSPORIDIUM

Analyses for *Giardia* cysts and *Cryptosporidium* oocysts were performed by BioVir Laboratories in accordance with the *ICR Microbial Laboratory Manual*, EPA/600/R-95/178, April 1996.

Appendix B-3 shows the results of selected weekly internal laboratory quality control samples (positive and negative samples) and monthly performance evaluation samples issued by USEPA. As shown in Appendix B-3, all results for these internal control samples and performance evaluation samples met quality control acceptance criteria.

The ICR Protozoan Method and CPMP Study protocol turbidity limit of 160 NTU for a filtered sample was exceeded by six samples, as listed in Table 2-3. These samples were not consistent with the ICR Study turbidity requirements.

Table 2-3.
Samples with Turbidity Limits Exceeded

Station	Sample ID	Sample Type (Liters filtered)	Sample Date	Turbidity (NTU)	Giardia (+/-)	Crypto (+/-)
BARKERNOBAY	D70123	Filtered (96L)	1/28/97	204	+	-
BARKERNOBAY	D80222	Filtered (100L)	2/24/98	230	+	-
DMC	D80218	Filtered (100L)	2/24/98	184	-	+
CLIFTON	D70100	Filtered (141L)	1/6/97	219	+	-
ARROYO	D61106	Filtered (96.9L)	11/17/96	718	-	-
ARROYO	D80221	Filtered (100L)	2/24/98	187	-	-

However, as discussed in this chapter (see Split Matrix Spike Recovery Study), and directly related to the poor precision and accuracy of this method, these samples were retained in the data set for this study. Samples D70123, D80222, and D70100 were positive for *Giardia*, with samples D61106 and D80221negative for both protozoans. Only sample D8218 was positive for *Cryptosporidium.* The poor recovery of the ICR Protozoan Method not only results in underestimation of actual concentrations of organisms present in a sample, but would also be expected to result in a greater number of non-detects at protozoan concentrations at or near the detection limit.

Since four of the samples were positive and did provide useful information on the presence of *Giardia* and *Cryptosporidium* at these sites which would otherwise be lost if

these data were excluded, they were included in the data set; the other samples with non-detects for both protozoa were also included for consistency. This approach is not incompatible with the overall goal of this study to obtain information on the pathogen status of the SWP and it's source waters, an approach which also allowed the use of non-ICR 4-liter grab samples when an ICR filtered sample was not able to be obtained. These four samples have been flagged in the data appendix should they need to be separated along with the grab samples, if only ICR comparable data are desired.

Split Matrix Spike Recovery Study

To determine the performance of the ICR Protozoan Method in the ambient waters sampled for this project, a recovery study was performed at five locations distributed throughout the project area. A second goal of this recovery study was to determine that the method's performance was consistent throughout the project area, which covered a distance of approximately 600 miles from the most northern sampling site at Alamar on the Sacramento River to Lake Perris, the terminal reservoir on the eastern Branch of the California Aqueduct in Southern California.

Changes in the physical and chemical nature of the water may occur as the water travels this distance, which also includes passage through not only the open channel California Aqueduct, but also several SWP reservoirs, tunnels, pipes, pumps, electrical power generating turbines, with additional water from the reservoir's watersheds also added to Aqueduct water. By conducting the split matrix spike recovery study using matrix water from throughout the project area, any gross changes in the performance of the method related to changes in the water matrix may be detected, at least to the extent of the ICR Protozoan Method's ability to detect such changes (USEPA 1996c, 1996d, 1997; Butler and Mayfield 1996; LeChevallier and Norton 1995; Jakubowski and others 1995).

The project area was sampled at five points along the SWP system and its source waters. In order to sample a range of turbidities and water matrices, matrix water was also collected from the American River (a component of the Sacramento River flow) which has relatively low turbidity. The turbidities at the other four sampling locations were generally higher than that of the American River, and represented the range of turbidity and matrix variability encountered throughout the project area of this study during any particular time interval.

The actual field protocol used to conduct the study is in Appendix C, and incorporated the following elements:

1. Matrix water of sufficient volume for two ICR protozoan samples (200+ liters) was collected in a polyethylene container, spiked with a certified number of *Giardia* cysts and *Cryptosporidium* oocysts (Appendix H), and kept well mixed using both mechanical and manual mixing devices during filtering.
2. Spike concentrations were 3,656 *Giardia* cysts and 4,480 *Cryptosporidium* oocysts in 200+ liters of matrix water. The formalin inactivated spike cysts and oocysts were obtained from ERA Labs in premeasured amounts.
3. Matrix water was maintained at <22°C to avoid spontaneous lysing of the oocysts, which occurs at approximately 30°C.
4. Two split samples were filtered according to ICR specifications by drawing a single stream of spiked and well mixed matrix water from the sampling container in through a single intake opening, which was then split and sent to two identically configured ICR filter assemblies arranged in parallel.
5. The sampling equipment was thoroughly cleaned using the field cleaning procedures in the USEPA ICR manual prior to performing the next matrix spike split. An additional detergent wash was added to the ICR procedure before the bleach rinse of the equipment specified in the ICR manual.

6. Blank split samples were collected using deionized water and all equipment used for the split samples to determine if spike cysts or oocysts were carried over from one split spiked sample set to another.
7. A standard filtered ICR protozoan sample was taken at the time of collection of the matrix water in order to determine the background concentrations of *Giardia* and *Cryptosporidium* in the matrix water.

As shown in Table 2-4, the recovery of spiked cysts and oocysts was much less than the minimum recovery expected by the USEPA for the ICR Study (USEPA 1996c, 1996d). The range of recoveries for the *Giardia* splits was <10 - 158 cysts/100 L, with an average recovery for all split samples of 46.3 cysts/100 L (2.53 percent). In one instance cysts were detected in the background sample with no cysts detected in one of the spiked samples. The background levels for the matrix water are included for reference, as is the standard deviation in parentheses in Table 2-4.

The recovery results for the *Cryptosporidium* splits are also shown in Table 2-4, and were also much less than the USEPA expected minimum recovery expected for the ICR Study (USEPA 1996c, 1996d). The range of recoveries for the *Cryptosporidium* splits was <4.5 - 24 oocysts/100 L, with an average recovery for all split samples of 7.75 oocysts/100 L (0.35 percent); 5 of 10 spiked samples had no oocysts detected. The background levels for the matrix water are included for reference on Table 2-4, as is the standard deviation in parentheses.

Table 2-4.

CPMP Split Matrix Spike Results

Matrix Water Source	*GIARDIA* Recovery 1828 cysts/spike		*Cryptosporidium* Recovery 2240 oocysts/spike		TURBIDITY
	cysts/100 L	% recovered	oocysts/100 L	% recovered	NTU
American River					2.6
Split Sample 1	5.0	0.27	<5	0	
Split Sample 2	6.7	0.37	<6.7	0	
Background	<6.7		<6.7		
San Joaquin River @ Vernalis					21.5
Split Sample 1	<10	0	<10	0	
Split Sample 2	33.7	1.84	<11.1	0	
Background	10		<10		
Banks PP at Bethany Reservoir					8.6
Split Sample 1	36	1.96	24	1.07	
Split Sample 2	45.5	2.50	22.6	1.01	
Background	<4.5		<4.5		
Devil Canyon					4.8
Split Sample 1	16.7	0.91	<8.3	0	
Split Sample 2	104.2	5.7	8.3	0.37	
Background	<6.3		<6.3		
California Aqueduct at Check 29					10.8
Split Sample 1	158	8.66	8.3	0.37	
Split Sample 2	57.1	3.12	14.3	0.64	
Background	<8.4		<8.4		
All Samples Average Recovery (SD_{n-1})[1]	**46.3** (50)	**2.53** (2.74)	**7.75** (9.59)	**0.35** (0.43)	
Positive Samples Only Average Recovery (SD_{n-1})[1]	**51.5** (50.2)	**2.81** (2.75)	**15.5** (7.55)	**0.69** (0.34)	

[1] Standard Deviation with n-1 degrees of freedom

Equipment Blanks

Equipment blanks were conducted using a total of 80 liters of distilled water, sending 40 liters to each filter apparatus. The equipment blanks were performed with the parallel sampling devices and all other equipment used to conduct the split matrix spike procedure. These blanks were run after three split matrix spike samples were filtered, and before the final two split matrix spike samples were filtered. The results for both splits were non-detect (Table 2-5), and indicate that no detectable spiked cysts or oocysts were carried over from one split matrix spike sample to another.

Table 2-5.

CPMP Split Matrix Spike Equipment Blank Results

Sample Type	*GIARDIA* cysts/100L	*Cryptosporidium* oocysts/100L	TURBIDITY NTU
Equipment Blank			<1
Sample 1	<2.7	<2.7	
Sample 2	<2.7	<2.7	

Conclusions

These results demonstrate that method performance is generally consistent with all water matrices obtained from within the project area. The low recoveries for both protozoa, the large standard deviations, along with the 50 percent non-detects for *Cryptosporidium* in spiked samples are indicative of the performance concerns related to the use of this method, and of the difficulties in interpreting the results obtained with it.

Although a detection limit goal of 10 cysts or oocysts per 100 L was specified and achieved for all but one of the 10 split matrix spike samples, and achieved or approached for most samples throughout the CPMP Study, this apparently had no effect on the ability of the ICR method to detect either the approximately 1,100 *Cryptosporidium* oocysts or 900 *Giardia* cysts spiked per sample (100 liters). Analyzing more of the sample by interpreting additional slides (5 slide maximum) to achieve a lower detection limit should increase the chance of detecting the protozoa if they are present. However, the calculated detection limit did not appear to indicate the actual performance of the method when recovery is low.

In the absence of other information regarding the performance of this method in the waters analyzed, the results obtained with the ICR Protozoan Method in this split spiked matrix study should be considered as an estimate of it's performance when interpreting the overall results of the CPMP Study. It should be noted that the analyzing laboratory for all CPMP samples had achieved and maintained USEPA ICR approval to participate in, and analyze samples for the ICR Study through analysis of ICR supplied monthly performance evaluation samples. As with any other ICR protozoan approved laboratory, at this time, the results provided by this laboratory have been accepted for the purposes of the ICR Study.

Direct Filter Spike Study

In January 1996, DWR's MWQI Program conducted a performance evaluation study using two laboratories and two protozoan methods (DWR 1996b). The August 1995 version of the USEPA ICR method was used by both BioVir and MWD laboratories, and a flow cytometry method was used only by BioVir. The cysts and oocysts were spiked directly onto the yarn wound filter, and matrix waters of three turbidities were then added to the container with the filter and submitted to the analyzing laboratories, with the results shown in Table 2-6. The wastewater

Table 2-6.

Direct Filter Spike Study[1]

GIARDIA	Cysts Seeded		2928 ± 447			
Matrix	MWD (8/95 ICR)		BioVir (8/95 ICR)		BioVir (Flow Cytometry)	
NTU	cysts/100L	%	cysts/100L	%	cysts/100L	%
60	350	11.9	1,266.7	43.3	233.3	7.97
10	232	7.92	1,220	41.7	110	3.76
Wastewater	90.4	3.09	1,733.3	59.2	166.7	5.69
CRYPTO	Oocysts Seeded		5532 ± 880			
Matrix	MWD (8/95 ICR)		BioVir (8/95 ICR)		BioVir (Flow Cytometry)	
NTU	oocysts/100L	%	oocysts/100L	%	oocysts/100L	%
60	440	7.97	33.3	0.60	166.67	3.01
10	200	3.6	<10	0	120	2.12
Wastewater	142.5	2.58	50	0.90	116.7	2.1

[1] Municipal Water Quality Investigations Program, Annual Report, Water Year 1995. August 1996. California Department of Water Resources, Division of Planning and Local Assistance.

matrix was obtained from a wastewater treatment plant, and was included in this study order to estimate the methods performance with water matrices of this type. Effluents from wastewater treatment plants are a source of protozoa.

By spiking the filters directly with the cysts and oocysts, any loss due to the spike passing through the filter was eliminated. As with the current ICR Study, a detection

limit was not specified. The spike cysts and oocysts were provide by Clancy Environmental Consultants (St. Albans, Vermont).

With the exception of the BioVir results for *Giardia*, which had very good recoveries for the ICR Protozoan Method, other recoveries were similar to those seen in the split spiked matrix study discussed previously, with only a small fraction of the spiked cysts and oocysts recovered. Recoveries for *Cryptosporidium* were consistently less than those for *Giardia*, with one sample having no spiked oocysts detected.

ICR PROTOZOAN METHOD PERFORMANCE

Background

The USEPA originally believed that the ICR Study objectives could be met if the laboratories analyzing for protozoa achieved an average of greater than 8 percent recovery for protozoan cysts. At this level of performance, the USEPA using simulation studies estimated that public water systems should be able to detect and count protozoa in two out of 18 monthly ICR water samples in at least 60 percent of the sites where protozoa were actually present (USEPA 1996c, 1996d).

A statistical adjustment factor would then be used to estimate the true protozoan concentrations from actual analytical results. This process would allow estimates of the different levels of treatment necessary to a achieve a specific finished water concentration of protozoa to be made on a national basis (USEPA 1996c). According to the USEPA, samples with *Cryptosporidium* not detected were to be used to help them determine how the sample volume analyzed and percent recovery affect the ability to quantify protozoa in source water.

The USEPA published an "ICR: Key Issues" paper (USEPA 1996d) in May 1996 just after the final ICR was promulgated (USEPA 1996c). This paper added detail to the questions raised regarding the ability of the method to provide meaningful data. The USEPA recognized that the ICR Protozoan Method is "...difficult to run, has poor recovery, and does not have a high level of precision."

A review of the method's performance resulted in the USEPA narrowing the scope of objectives for the ICR Study. The USEPA no longer believed, based on statistical analysis, that the ICR Protozoan Method could be used to produce site-specific information which public water systems could use to comply with future rules (USEPA 1996d). As stated by the USEPA, (USEPA 1999) "Experience with the ICR Protozoan Method has shown that is usually underestimates the levels and occurrence of *Giardia sp.* and *Cryptosporidium sp.*, the two protozoan parasites that it was designed to detect."

However the USEPA believed the ICR Protozoan Method should be used in the ICR Study for the following reasons (USEPA 1996d):

- There is good likelihood it will provide useful data.
- The more experience laboratories have with the method, the better their performance should be.
- Through subsequent testing, an adjustment factor may be generated to improve the utility of rule-generated protozoan data.
- There is a public perception that protozoa occurrence are a significant health issue; whatever data can be gathered will help address this concern.
- The total cost of including it is less than $5 million of the $130 million estimated for the entire rule, with less than $1 million attributed to the incremental inclusion of *Cryptosporidium* over *Giardia*.

Performance

The ICR Protozoan Method's performance is subject to limitations at all steps in the procedure. The wound yarn filter cartridge used has a nominal pore size of 1 micron, and this nominal porosity can allow cysts, and particularly oocysts to pass through. Due to the adhesive properties of the *Cryptosporidium* oocysts, once captured on the filter, they are very difficult to remove (elute) for analysis. A density gradient Percoll-sucrose solution is used during the purification procedure to separate the organisms from other debris in the sample, but other objects with the same specific gravity as the cysts and oocysts, e.g., algae, will also be isolated. Some of these co-separated objects may interfere with the staining and examination of slide mounted specimens. The antibodies for *Giardia* and *Cryptosporidium* may cross react with some yeast, algal cells, and invertebrate eggs, and some particles may auto-fluoresce, all of which could lead to false positives if they were counted. The method is also tedious and time-consuming (USEPA 1997b; Butler and Mayfield 1996).

As shown in the split spiked matrix study above, and in other studies, both the accuracy and precision of the ICR Protozoan Method are poor (USEPA 1996c, 1996d, 1997b; LeChevallier and Norton 1995; Jakubowski and others 1995; Klonicki 1997). Results from two round robin studies conducted by the USEPA determined mean recovery efficiencies for *Giardia* of 25 and 44 percent (range 0-139 percent), with *Cryptosporidium* mean recoveries of 23 and 35 percent (range 0-140 percent) (Jakubowski and others 1995). Using 58 simulated raw water samples with sample concentrates added to tap water to produce a 150 NTU matrix, LeChevallier and Norton (1995) determined geometric means for recovery of *Giardia* of 42.4 percent (range 18.2-118.3 percent) and for *Cryptosporidium* of 23.6 percent (range 8.7-74.7 percent).

The USEPA (1998) created a laboratory spiking program, which could result in the development of an "aggregate recovery rate adjustment factor" to be used by ICR

labs performing analyses using the ICR Protozoan Method, an approach which is not universally accepted. The spiking program included water treatment plants participating in the ICR Study and the labs that analyzed their samples. On separate sampling dates an additional 100 L of raw water was sent, along with the ICR protozoan sample collected, to an EPA contract lab where it was spiked and filtered. The spiked sample filter was then sent to the submitting water treatment plant's ICR laboratory for analysis using the current ICR method.

The results will be used by the USEPA to interpret ICR Protozoan Method results for raw water, and may be used to adjust estimated national protozoan concentrations when evaluating regulatory options. This spiked matrix recovery study approach was similar to the split spiked matrix recovery study conducted for the CPMP Study, and should yield information on how the current ICR Protozoan Method performed in the field with actual raw water compared with in the laboratory where recovery was determined through the use of monthly performance evaluation samples in a deionized water matrix.

USEPA Method 1622

The USEPA recently developed Method 1622 for use in detecting *Cryptosporidium* and *Giardia* in water, which has been undergoing round-robin testing (*Cryptosporidium* only for now, *Giardia* to follow) (USEPA 1998a, 1998b). This method was designed to improve the recovery, precision, and detection limit for both *Cryptosporidium* and *Giardia*. The method is designed for use in aqueous matrices, and employs filtration, immunomagnetic separation, along with immunofluorescence assay and confirmation using vital dye staining and differential interference contrast microscopy (USEPA 1997a). A method detection limit of 4 oocysts/liter using a 10 liter sample was expected when no interferences are present.

Chapter 3

RESULTS AND DISCUSSION

The results of the 248 samples collected and analyzed for this sampling program are included in this discussion. The monthly, storm and flood event sampling results are reported separately for comparative purposes, and are also combined with each other for an overall view of all study results.

All *Giardia* and *Cryptosporidium* results discussed in this report are based on total IFA counts. The total IFA count is the sum of the empty, amorphous structure, and internal structure counts which results from analysis of the protozoan sample using the ICR method. The total count includes structures which are known to be non-viable, and is intended to account for all structures which could be classified as cysts and oocysts according to the ICR method protocol, and represents a conservative use of the data. The method is not intended to differentiate viable cysts or oocysts from non-viable ones.

GIARDIA AND *CRYPTOSPORIDIUM*

Summary Results

The summary results for *Giardia* and *Cryptosporidium* are shown in Table 3-1, along with results from the LeChevallier and Norton (1995) and MWD (1993) studies.

Table 3-1.

***Giardia* and *Cryptosporidium* Summary Statistics for Phase I**

(Total IFA count)

Study		*Giardia* (Cysts/100L)			*Cryptosporidium* (Oocysts/100L)			
		Number Positive	Range	Geo. Mean	Number Positive	Range	Geo. Mean	N
CPMP Monthly	Phase I[a]	22% (35 of 158)	2.4 - 92.3	16.6	4% (6 of 158)	9.0 - 26.7	18.0	158
	Phase II[b]	30% (11 of 37)	2.5 - 62.8	15.6	3% (1 of 37)	13.3	N/A	37
	Combined	24% (46 of 195)	2.4 - 92.3	16.4	4% (7 of 195)	9.0 - 26.7	17.2	195
CPMP Event	Phase I	33% (9 of 27)	10.05 - 129.8	58.9	30% (8 of 27)	4.4 - 200	35.0	27
	Phase II	35% (9 of 26)	10 - 140	28.5	19% (5 of 26)	10 - 50	22.6	26
	Combined	34% (18 of 53)	10 - 140	40.9	24% (13 of 53)	4.4 - 200	29.6	53
CPMP Combined	Phase I	24% (44 of 185)	2.4 - 129.8	21.5	8% (14 of 185)	4.4 - 200	26.3	185
	Phase II	32% (20 of 63)	2.5 - 140	20.4	10% (6 of 63)	10 - 50	20.7	63
	Combined	26% (64 of 248)	2.4 - 140	21.2	8% (20 of 248)	4.4 - 200	24.5	248
MWD 1993		12% (6 of 48)	6 - 82	20	35% (17 of 48)	5 - 132	32	48
L & N 1995		54% (187 of 347)	2 - 4,380	200	60% (209 of 347)	6.6 - 6,510	240	347

[a]Phase I: October 1996 through October 1997
[b]Phase II: November 1997 through April 1998

Both of these studies used an earlier version or versions of an immunofluorescence antibody (IFA) method very similar to the USEPA ICR IFA method used for both the CPMP and the ICR studies.

The LeChevallier and Norton (1995) study reflects the results of 347 surface water samples collected between 1988 and 1993 from 72 water treatment plants in 15 states and two Canadian provences. The average sample size for this study was 499 L, with a range of 86.6 to 3,394 L; this average is significantly larger than the 100L

specified by the ICR IFA method used for the CPMP Study. A larger filtered sample volume generally produces greater analytical sensitivity with this method. Most samples were obtained from water treatment plants in the eastern United States and Canada, although some were from the western U.S.

The MWD (1993) study was conducted by MWD in 1992-1993, and used an IFA method (ASTM 1992) similar to the USEPA ICR method. Sampling locations included three sites in the Sacramento-San Joaquin Delta, i.e., Greene's Landing, Banks Pumping Plant, and the Delta-Mendota Canal, and one site at Check 29 in the California Aqueduct. All four of these sites were included in the CPMP Study. While a detection limit was not specified, the detection limits for this study ranged from <2 to <126 cysts or oocysts/100L.

The results are reported for the two sampling periods comprising the CPMP Study. Phase I of the study covered the first year of sampling (October 1996 through October 1997) at all sampling stations. Phase II comprised the last 6 months of study (November 1997 through April 1998) at a reduced number of sampling stations selected based on the frequency of detection seen in Phase I of the study.

Giardia

The range of positive monthly CPMP *Giardia* results was 2.4 to 92.3 cysts/100L, with a geometric mean of 16.4 cysts/100L (Table 3-1). The LeChevallier and Norton (1995) Study had a range of 2 - 4380 cysts/100L for *Giardia*, and a geometric mean of 200 cysts/100L, with the MWD (1993) Study having a range of 6-82 cysts/100L and a geometric mean of 20.

Both the range and geometric mean of the CPMP event samples were higher than the CPMP monthly samples. The detection frequency for the CPMP event

samples (34 percent) was higher than that of the monthly samples (24 percent), with the MWD Study (13 percent) somewhat lower.

Cryptosporidium

The range of positive monthly CPMP *Cryptosporidium* results was 9.0 to 26.7 oocysts/100L, with a geometric mean of 17.2 oocysts/100L (Table 3-1). The LeChevallier and Norton (1995) Study had a range of 6.6 to 6,510 oocysts/100L for *Cryptosporidium*, and a geometric mean of 240 oocysts/100L, with the MWD (1993) Study having a range of 5-132 oocysts/100L and a geometric mean of 32 oocysts/100L. Both the range and geometric mean of the CPMP event samples were higher than that of the CPMP monthly samples. The *Cryptosporidium* detection freuency for the CPMP event samples (35 percent) was higher than that of the monthly samples (4 percent), with the MWD Study (35 percent) somewhat higher.

Giardia and *Cryptosporidium* Results by Station

The results for both *Giardia* and *Cryptosporidium* for selected sampling locations are displayed as the number of positive and negative samples In Figures 3-1 and 3-2. These data are shown in tabular form in Appendix D as monthly and storm event

Figure 3-1. *Giardia* Percent Positive - Monthly and Event Samples Combined

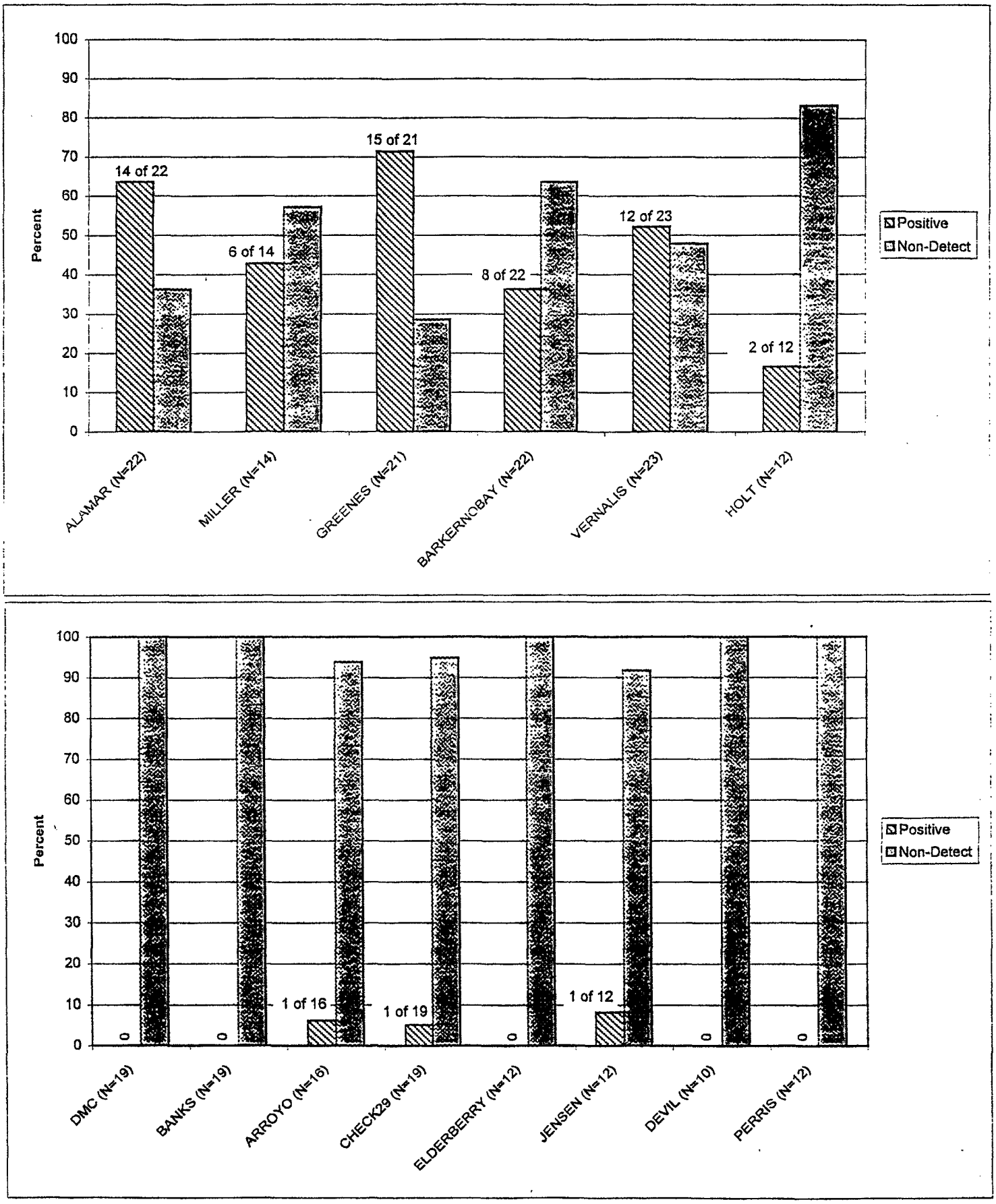
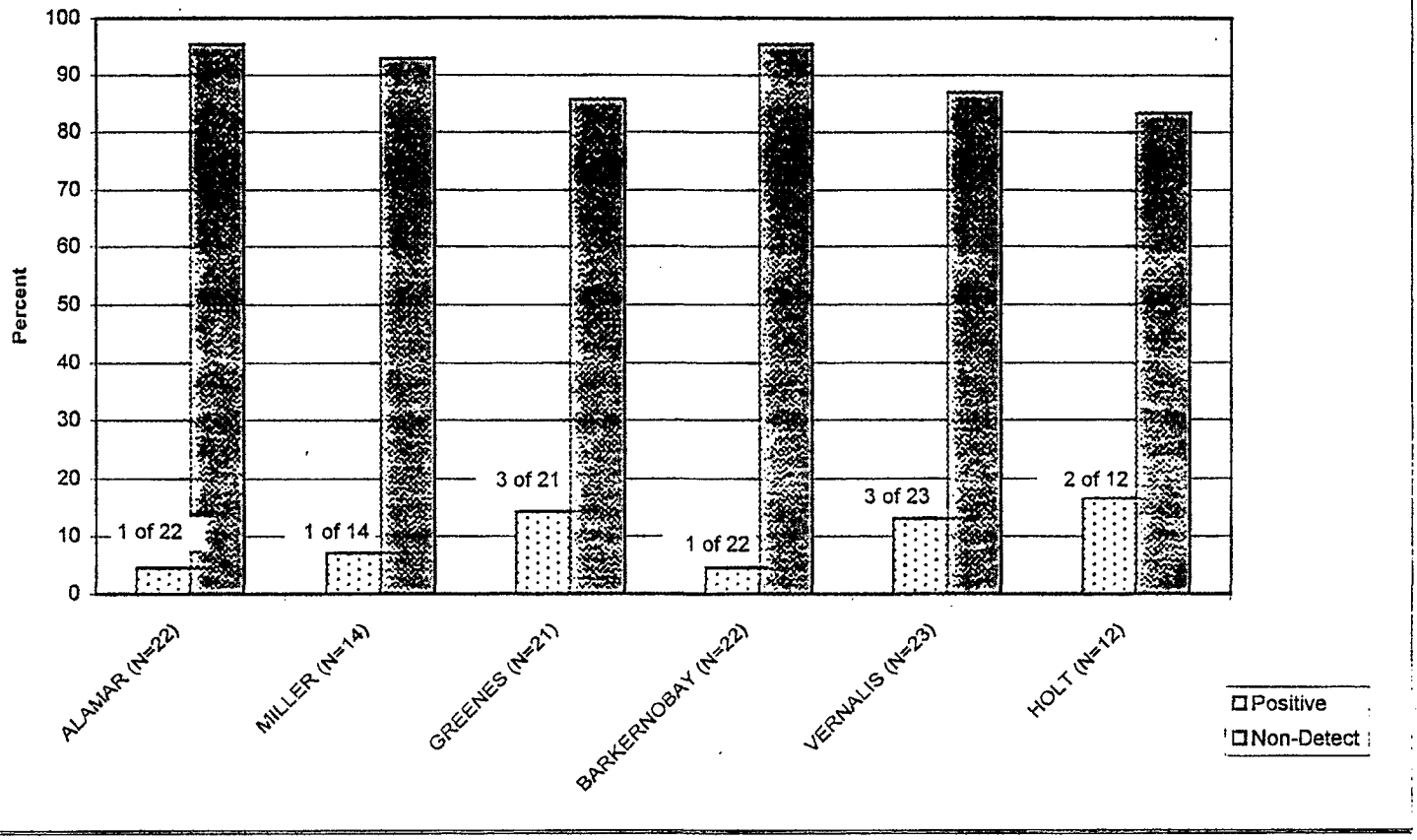
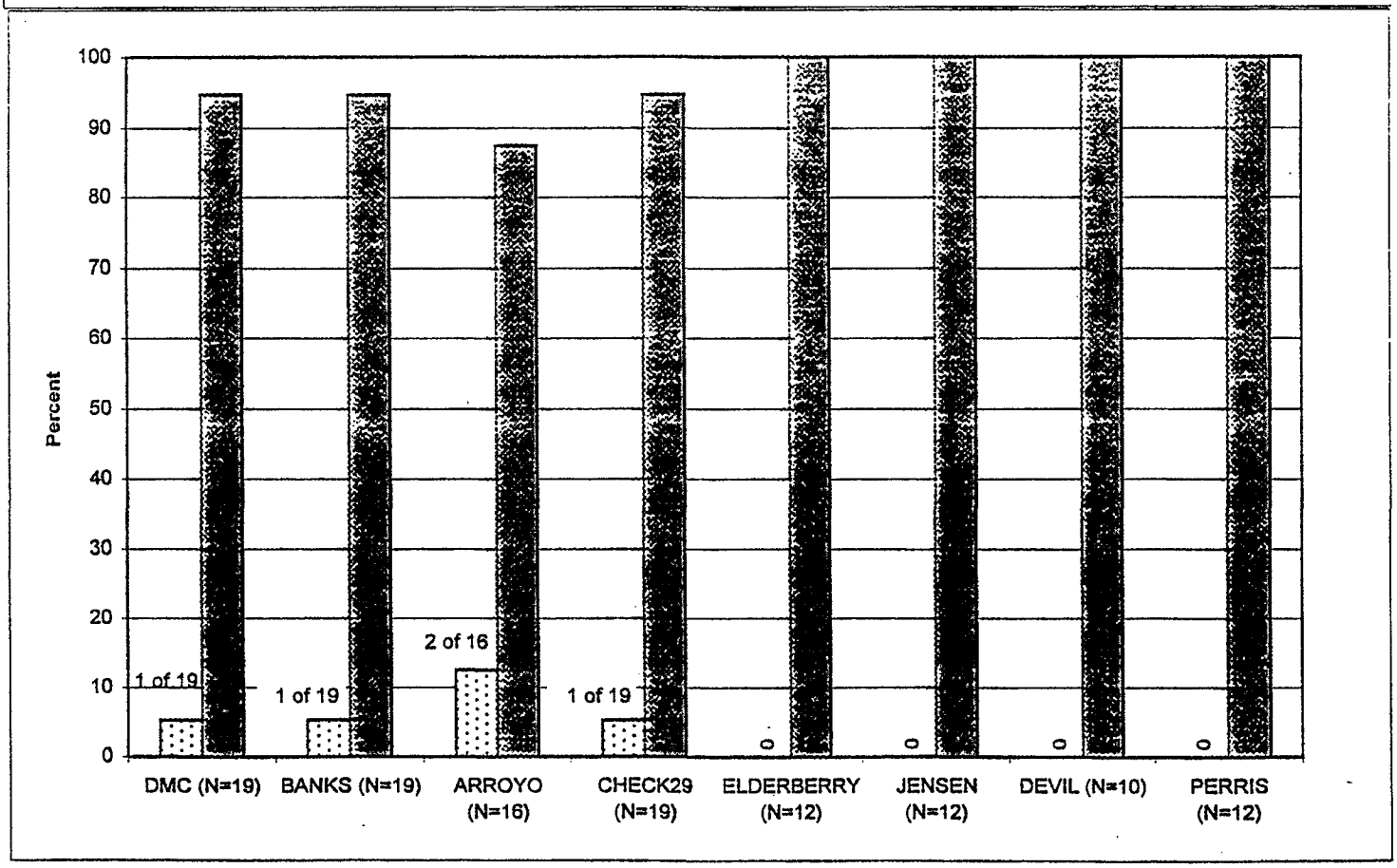


Figure 3-2. Cryptosporidium Percent Positive - Monthly and Event Samples Combined





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samples combined, monthly, and storm event sample results. Also displayed are the range and geometric mean (where applicable) for each station, along with the average detection limit and range of detection limits for each station for both positive and negative samples.

Giardia

The detection frequency for both *Giardia* and *Cryptosporidium* is shown as the combined results for the monthly and storm event samples for each sampling location in Figures 3-1, with additional details provided in Table 3-2. The highest detection frequencies for *Giardia* for stations with either monthly or monthly and event samples were found in the Sacramento and San Joaquin rivers which are the source waters of the SWP.

Beginning at Alamar (64 percent), the most northern Sacramento River site, the detection frequency decreased at Miller Park site (43 percent), which is downstream of the confluence of the Sacramento and American rivers, and downstream of a relatively minor City of Sacramento wastewater/storm water discharge. The frequency again increased at the Sacramento River Greenes Landing site (71 percent), which is approximately 10 miles downstream of the cities of Sacramento's (approximately 150 MGD) (CUWA 1995) and West Sacramento's (approximately 5.4 MGD) (Tabun 1998) major waste water discharges. Greenes Landing had the highest *Giardia* detection frequency of any sampling location in the CPMP Study.

Giardia detection frequency at the San Joaquin River site at Vernalis (52 percent), which is upstream of the City of Stockton's major wastewater discharge, was much higher than the frequency at the San Joaquin River Holt site (17 percent), which is downstream of the City of Stockton's wastewater discharge. *Giardia* detection frequency at the North Bay Aqueduct intake located at the Barker Slough Pumping

Table 3-2. *Giardia* and *Cryptosporidium* Combined Summary Statistics by Station

		Giardia (cysts/100L)					*Cryptosporidium* (oocysts/100L)				
		No. of Samples	Geo. Mean	Range	Average Detection Limit	Range of Detection Limits	No. of Samples	Geo. Mean	Range	Average Detection Limit	Range of Detection Limits
ALAMAR	Pos.	64% (14 of 22)	22.1	6.7 - 86.5	11.5	3.3 - 25.7	5% (1 of 22)	N/A	20	N/A	20
	Neg.	36% (8 of 22)			9.6	5 - 14.5	95% (21 of 22)			10.3	3.3 - 25.7
MILLER	Pos.	43% (6 of 14)	25.9	6.6 - 100	25.2	5 - 100	7% (1 of 14)	N/A	11.11	N/A	11.11
	Neg.	57% (8 of 14)			9.0	2.4 - 26.6	93% (13 of 14)			16.3	2.4 - 100
GREENES	Pos.	71% (15 of 21)	18.6	2.5 - 140	9.7	2.5 - 25	14% (3 of 21)	19.4	17.8 - 20.4	12.7	10 - 17.8
	Neg.	29% (6 of 21)			7.1	3.3 - 11.8	86% (18 of 21)			8.3	2.5 - 25
SHAG	Pos.	0 of 1	N/A	N/A	N/A	N/A	1 of 1	N/A	200	N/A	200
	Neg.	1 of 1			N/A	200	0 of 1			N/A	N/A
BARKERNOBAY	Pos.	36% (8 of 22)	27.6	10.05 - 75	22.1	10.05 - 40	5% (1 of 22)	N/A	20.1	N/A	10.05
	Neg.	64% (14 of 22)			29.4	9.45 - 99	95% (21 of 22)			27.5	9.45 - 99
MOKELUMNE	Pos.	1 of 1	N/A	25	N/A	12.5	0 of 1	N/A	N/A	N/A	N/A
	Neg.	0 of 1			N/A	N/A	1 of 1			N/A	12.5
VERNALIS	Pos.	52% (12 of 23)	21.7	9.4 - 125	14.8	9.4 - 30.8	13% (3 of 23)	25.7	13.3 - 62.5	13.0	10.2 - 15.6
	Neg.	48% (11 of 23)			84.7	11.7 - 333.3	87% (20 of 23)			53.5	9.4 - 333.3
HOLT	Pos.	17% (2 of 12)	N/A	10 - 26.7	11.7	10 - 13.3	17% (2 of 12)	N/A	17 - 26.7	17.6	8.5 - 26.7
	Neg.	83% (10 of 12)			12.4	8.5 - 26.7	83% (10 of 12)			11.2	9.3 - 15.8
CLIFTON	Pos.	40% (2 of 5)	N/A	16.7 - 129.8	40.8	16.7 - 64.9	20% (1 of 5)	N/A	33.3	N/A	16.7
	Neg.	60% (3 of 5)			19.0	10.4 - 36	80% (4 of 5)			30.45	10.4 - 64.9

Table 3-2. *Giardia* and *Cryptosporidium* Combined Summary Statistics by Station *(Continued)*

		Giardia (cysts/100L)					*Cryptosporidium* (oocysts/100L)				
		No. of Samples	Geo. Mean	Range	Average Detection Limit	Range of Detection Limits	No. of Samples	Geo. Mean	Range	Average Detection Limit	Range of Detection Limits
KERN	Pos.	1 of 1	N/A	73	N/A	10.4	1 of 1	N/A	10.4	N/A	10.4
	Neg.	0 of 1			N/A	N/A	0 of 1			N/A	N/A
DMC	Pos.	0 of 19	N/A	N/A	N/A	N/A	5% (1 of 19)	N/A	50	N/A	25
	Neg.	19 of 19			18.4	7.3 - 78.8	95% (18 of 19)			18.0	7.3 - 78.8
BANKS	Pos.	0 of 19	N/A	N/A	N/A	N/A	5% (1 of 19)	N/A	168.9	N/A	33.8
	Neg.	19 of 19			9.4	2.6 - 33.8	95% (18 of 19)			8.0	2.6 - 15.2
ARROYO	Pos.	6% (1 of 16)	N/A	2.4	N/A	2.4	12% (2 of 16)	N/A	10 - 103.2	17.9	10 - 25.8
	Neg.	94% (15 of 16)			15.9	1.6 - 103	88% (14 of 16)			14.7	1.6 - 103
KERNSWP	Pos.	0 of 1	N/A	N/A	N/A	N/A	0 of 1	N/A	N/A	N/A	N/A
	Neg.	1 of 1			N/A	8.9	1 of 1			N/A	8.9
CHECK29	Pos.	6% (1 of 18)	N/A	9	N/A	9	6% (1 of 18)	N/A	9	N/A	9.0
	Neg.	94% (17 of 18)			10.1	7.8 - 20	94% (17 of 18)			10.1	7.8 - 20
ELDERBERRY	Pos.	0 of 12	N/A	N/A	N/A	N/A	0 of 12	N/A	N/A	N/A	N/A
	Neg.	12 of 12			7.0	2.2 - 11	12 of 12			7.0	2.2 - 11
PIRU	Pos.	0 of 1	N/A	N/A	N/A	N/A	0 of 1	N/A	N/A	N/A	N/A
	Neg.	1 of 1			N/A	31.25	1 of 1			N/A	31.25
ELIZABETH	Pos.	0 of 1	N/A	N/A	N/A	N/A	0 of 1	N/A	N/A	N/A	N/A
	Neg.	1 of 1			N/A	1.3	1 of 1			N/A	1.3

Table 3-2. *Giardia* and *Cryptosporidium* Combined Summary Statistics by Station *(Continued)*

		Giardia (cysts/100L)					*Cryptosporidium* (oocysts/100L)				
		No. of Samples	Geo. Mean	Range	Average Detection Limit	Range of Detection Limits	No. of Samples	Geo. Mean	Range	Average Detection Limit	Range of Detection Limits
MILLCR	Pos.	0 of 3	N/A	N/A	N/A	N/A	33% (1 of 3)	N/A	4.4	N/A	4.4
	Neg.	3 of 3			5.8	4.4 - 7.3	67% (2 of 3)			6.55	5.8 - 7.3
JENSEN	Pos.	8% (1 of 12)	N/A	4.11	N/A	4.11	0 of 12	N/A	N/A	N/A	N/A
	Neg.	92% (11 of 12)			6.0	1.18 - 10.2	12 of 12			5.8	1.18 - 10.2
MILLS	Pos.	0 of 1	N/A	N/A	N/A	N/A	0 of 1	N/A	N/A	N/A	N/A
	Neg.	1 of 1			N/A	16.51	1 of 1			N/A	16.51
DEVIL	Pos.	0 of 10	N/A	N/A	N/A	N/A	0 of 10	N/A	N/A	N/A	N/A
	Neg.	10 of 10			8.4	1.3 - 13.6	10 of 10			8.4	1.3 - 13.6
PERRIS	Pos.	0 of 12	N/A	N/A	N/A	N/A	0 of 12	N/A	N/A	N/A	N/A
	Neg.	12 of 12			10.3	1.1 - 43.9	12 of 12			10.3	1.1 - 43.9

[1] Not applicable.

Plant was 36 percent positive, which was less than that seen at the Sacramento River sites or the Vernalis site on the San Joaquin River, but higher than the levels seen within the SWP. The majority of results from samples collected from sampling locations either in the California Aqueduct or in the SWP reservoirs were below the detection limit.

The Banks Pumping Plant site represents water that has entered the SWP at the intake point in Clifton Court, and the Clifton site (at the radial gates in Clifton Court) represents storm water flows into Clifton Court. Neither *Giardia* nor *Cryptosporidium* were detected at Banks Pumping Plant, and only one of three storm water samples from the Clifton site was positive for *Cryptosporidium*.

Giardia detection was more frequent in the river source waters of the SWP compared with the sampling locations within the SWP system itself, including the reservoirs. The majority of results from samples collected from sampling locations either in the California Aqueduct or in the SWP reservoirs were below the detection limit.

Cryptosporidium

Cryptosporidium detection frequencies were relatively low compared with those of *Giardia*, which should be considered along with the fact that recovery of *Cryptosporidium* was less than that for *Giardia* in this study (see Chapter 2). While *Cryptosporidium* was present at all Sacramento River, San Joaquin River, North Bay Aqueduct, and Banks Pumping Plant sampling locations, frequencies within the SWP system were uniformly low (Table 3-2, Figure 3-2). The highest detection frequency in the CPMP Study for stations with either monthly or monthly and event samples (17 percent) was at the Holt Road sampling site on the San Joaquin River.

As with *Giardia*, *Cryptosporidium* detection was more frequent in the river source waters of the SWP compared with the sampling locations within the SWP system itself, including the reservoirs. For stations where both *Giardia* and *Cryptosporidium* were detected, the percent of samples positive for *Giardia* was generally much higher than for *Cryptosporidium*, a finding which may be influenced by the method performance.

Giardia and *Cryptosporidium* Seasonal Results

The detection frequency, geometric mean, and range of positive results for both protozoans were greater in the storm and flood event samples compared with the monthly samples (Table 3-1). While some storm event samples were collected at locations different from the monthly sites (Tables 1-1 and 1-2) in order to characterize watershed or other local inputs to the system, other storm event sample locations were the same as the monthly sampling locations. The results by station for the monthly samples separated from the flood and storm event samples are shown in Appendix D.

In order to compare the *Giardia* and *Cryptosporidium* results in wet and dry seasons, the CPMP data set was divided into several subsets. Since the CPMP Study period of 18 months covered two wet seasons and one dry season, it was possible to compare two wet season data sets with one dry season data set. The two wet season data sets include all results for the period October 1996 through March 1997 (N=161) and October 1997 through April 1998, with the dry season including results for the period of April 1997 through September 1997 (N=87).

The Phase I wet season had 29 percent positive samples compared with Phase II which had 35 percent positive samples. The overall detection frequency was 31 percent for both wet season data sets combined. The Phase II wet season data reflect results from only the sampling locations from Check 29 north, and was

influenced by SWP source waters having higher detection frequencies relative to samples from within the SWP system, which are contained in the data set for the Phase I results.

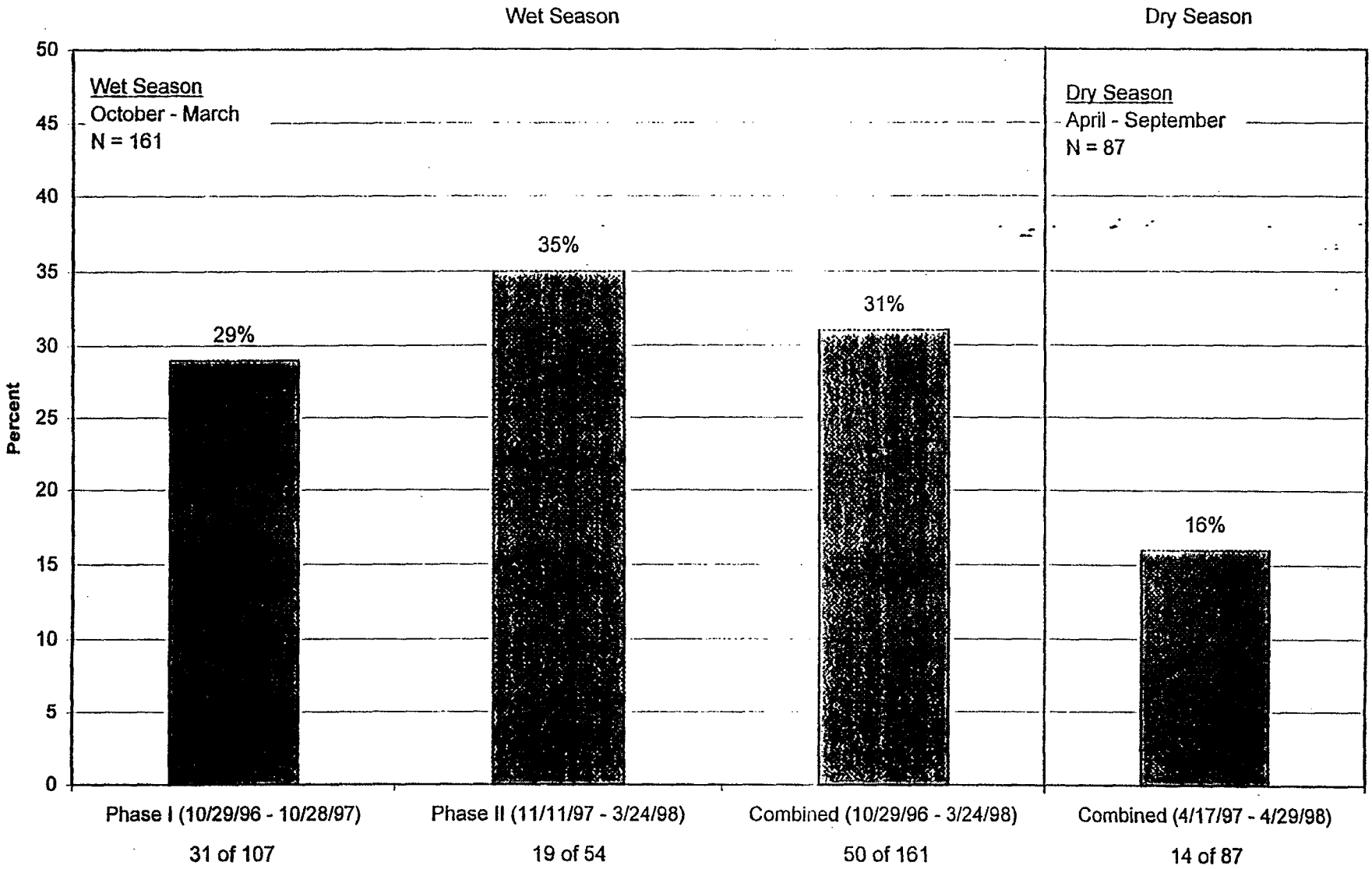
Both *Giardia* and *Cryptosporidium* were detected more frequently in both sets of wet season samples relative to the dry season samples (Figures 3-3 and 3-4). The wet season samples contained the majority of the storm and flood event samples, which as previously discussed, had both a greater detection frequency and a higher geometric mean for positive samples compared with the monthly samples (Table 3-1).

Giardia and *Cryptosporidium* in Source and SWP Waters

The CPMP data set was divided into subsets, one representing sampling locations in the source waters (N=122), and the other the sampling locations located within the SWP system of aqueducts, pipes, tunnels, and reservoirs (N=126). Source water sampling locations included those samples from locations which are the principal source waters of the SWP system, primarily the Sacramento and San Joaquin rivers. The sampling locations included in either the source or SWP subsets are shown in Table 3-3.

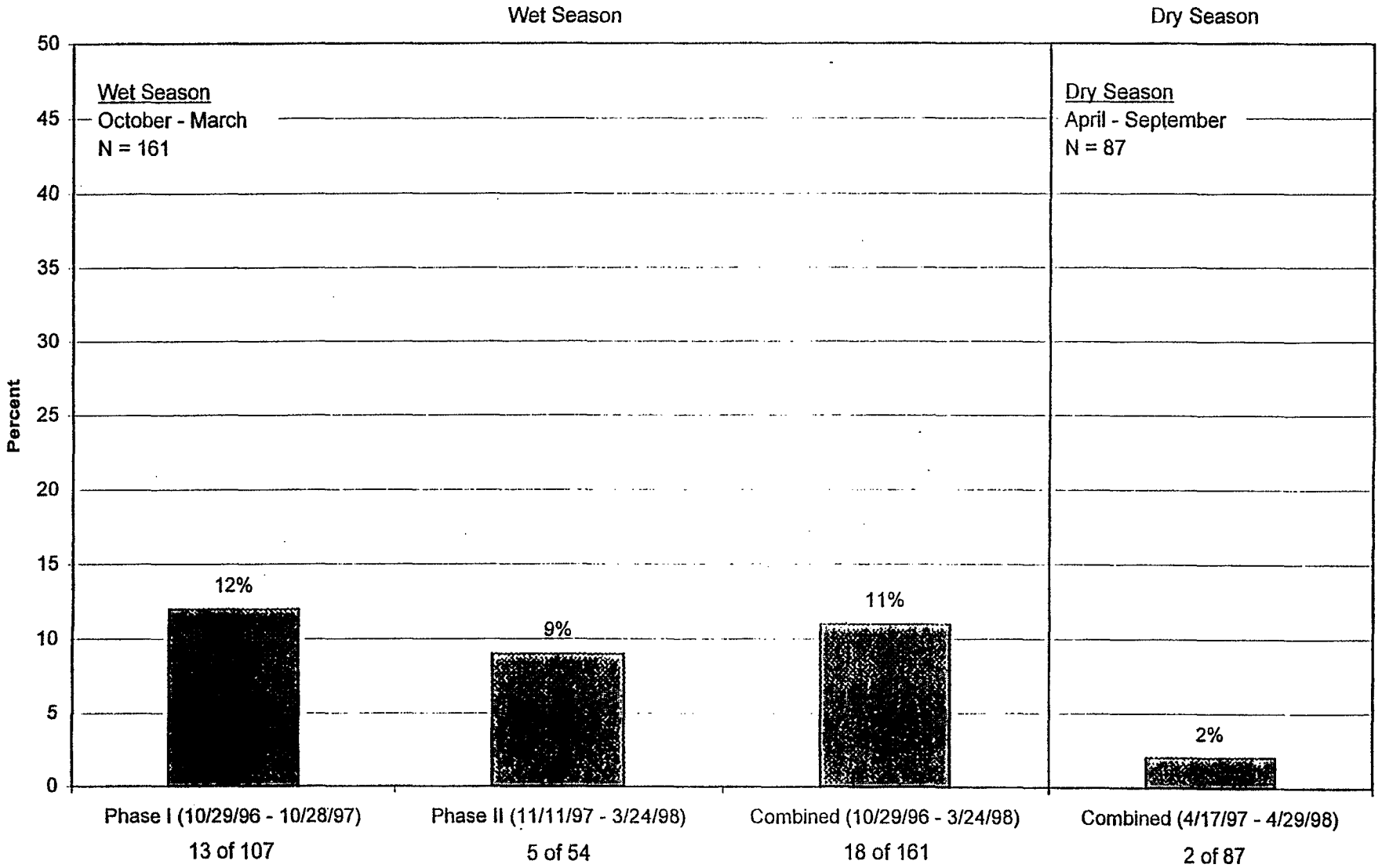
Each subset includes all samples collected for the sampling location, including storm and flood event samples combined with the monthly samples. For the SWP locations, the samples collected for storm events from sampling locations located at either the aqueduct/reservoir or the watershed inputs to the aqueduct/reservoir were included. For example, in addition to the monthly sampling location which represents water from the reservoir, a storm event sampling location for Pyramid Lake was located on Piru Creek, which is the major watershed input to this SWP reservoir and was included in the SWP subset.

Figure 3-3. Positive *Giardia* Samples - Seasonal



3-41

Figure 3-4. Positive *Cryptosporidium* Samples - Seasonal



GiardiaCryptoDataforPhase1and2.xls

Fig34

Table 3-3.

Source and SWP Monitoring Locations

Source Stations	SWP Stations
Sacramento River	Banks Pumping Plant
Alamar Marina	Delta Mendota Canal
Miller Park	Lake Del Valle - Arroyo Valle Creek
Greenes Landing	California Aqueduct - Check 29
San Joaquin River	California Aqueduct at Kern Intertie
Vernalis	Pyramid Lake
Holt Road	Castaic Lake - Jensen WTP
NBA - Barker Slough Pumping Plant	Silverwood Lake - Mills WTP
Shag Slough - Liberty Island	Devil Canyon
Mokelumne River - New Hope	Lake Perris
Kern River	
Clifton Court - Radial Gates	
Total samples = 122	**Total Samples = 126**

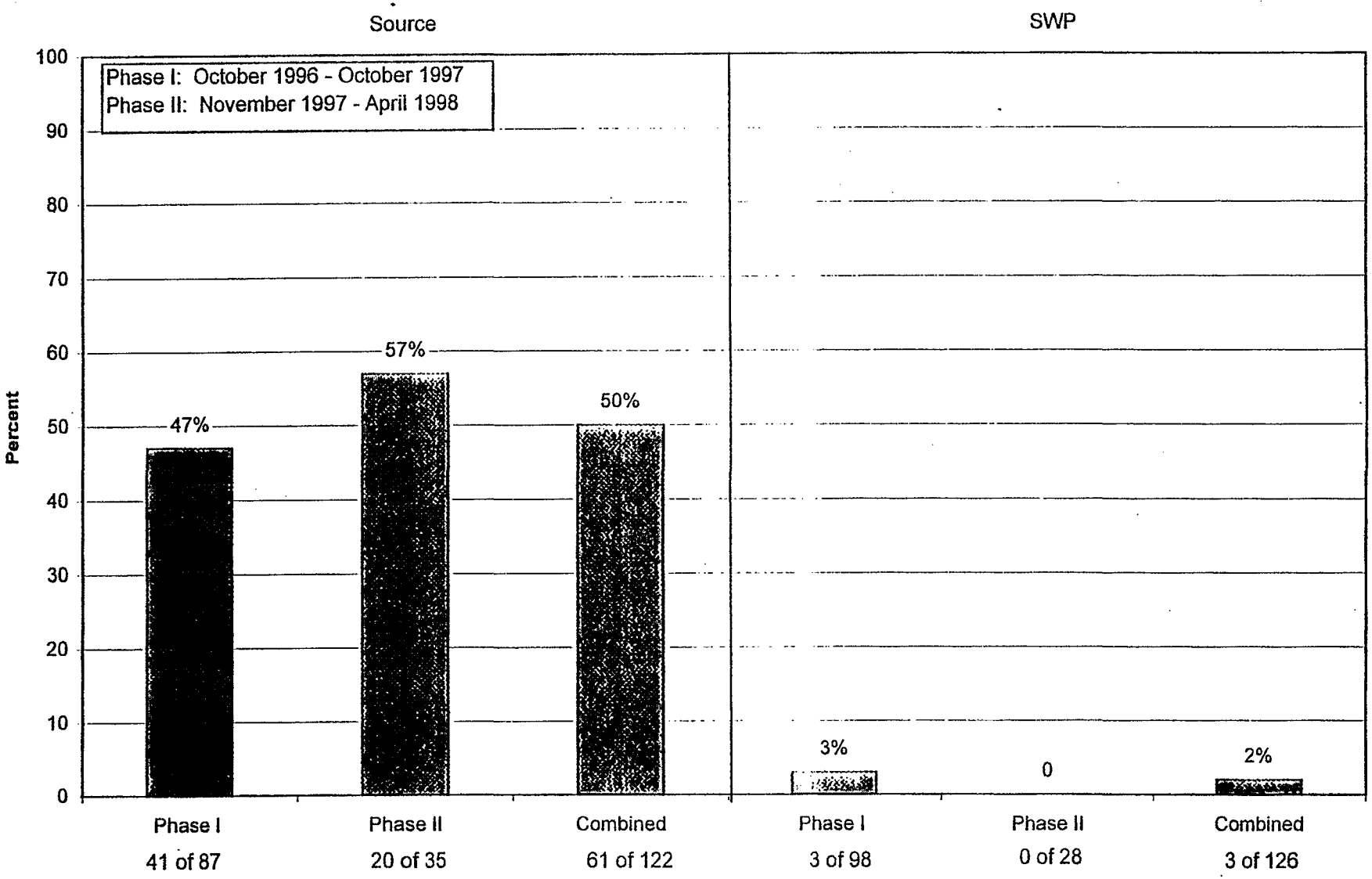
A difference was observed in the both this CPMP Study (Figures 3-1, 3-2, and Table 3-2), and to a limited extent in the MWD (1993) Study with regard to the detection frequency and geometric mean of positive samples in the source waters of the SWP compared to the water in the SWP system. The CPMP Study results indicate a higher frequency of detection and a higher geometric mean in positive samples from the SWP source waters compared to samples from waters within the SWP itself.

The detection frequency of the protozoans in source waters is compared to SWP waters in Figures 3-5 and 3-6 as the percent of positive samples. *Giardia* was detected in 50 percent of the source water samples and in only 3 percent of the SWP samples. *Cryptosporidium* was present in 11 percent of the source water samples and in 5 percent of the SWP samples. The percent positive data by station for monthly and event samples combined (Tables 3-1 and 3-2), and data grouped by SWP or source (Figures 3-5 and 3-6), show this difference in both detection frequency and concentration of organisms as water travels from the source waters of the Sacramento and San Joaquin rivers, through the Delta and California Aqueduct, to the terminal reservoirs of the SWP system.

The reasons for this difference may be related to the fate processes at work throughout the SWP system study area capable of affecting cyst and oocyst concentrations. These fate processes are not well defined for these organisms. The MWD (1993) Study found that pathogen concentrations, which included enteric viruses and coliforms, decreased when results from sampling locations in the Sacramento River and Delta were compared with results from the California Aqueduct Check 29 sampling location (Kern County).

As water moves through the SWP system it is exposed to passage through various pumps, power generation turbines, pipes, open channels, forebays, afterbays, and reservoirs, all of which have unknown effects on the cysts and oocysts. Fate processes which could remove cysts and oocysts from the water column likely include both settling in the aqueduct and reservoirs, natural decay, or other physical or biological environmental processes.

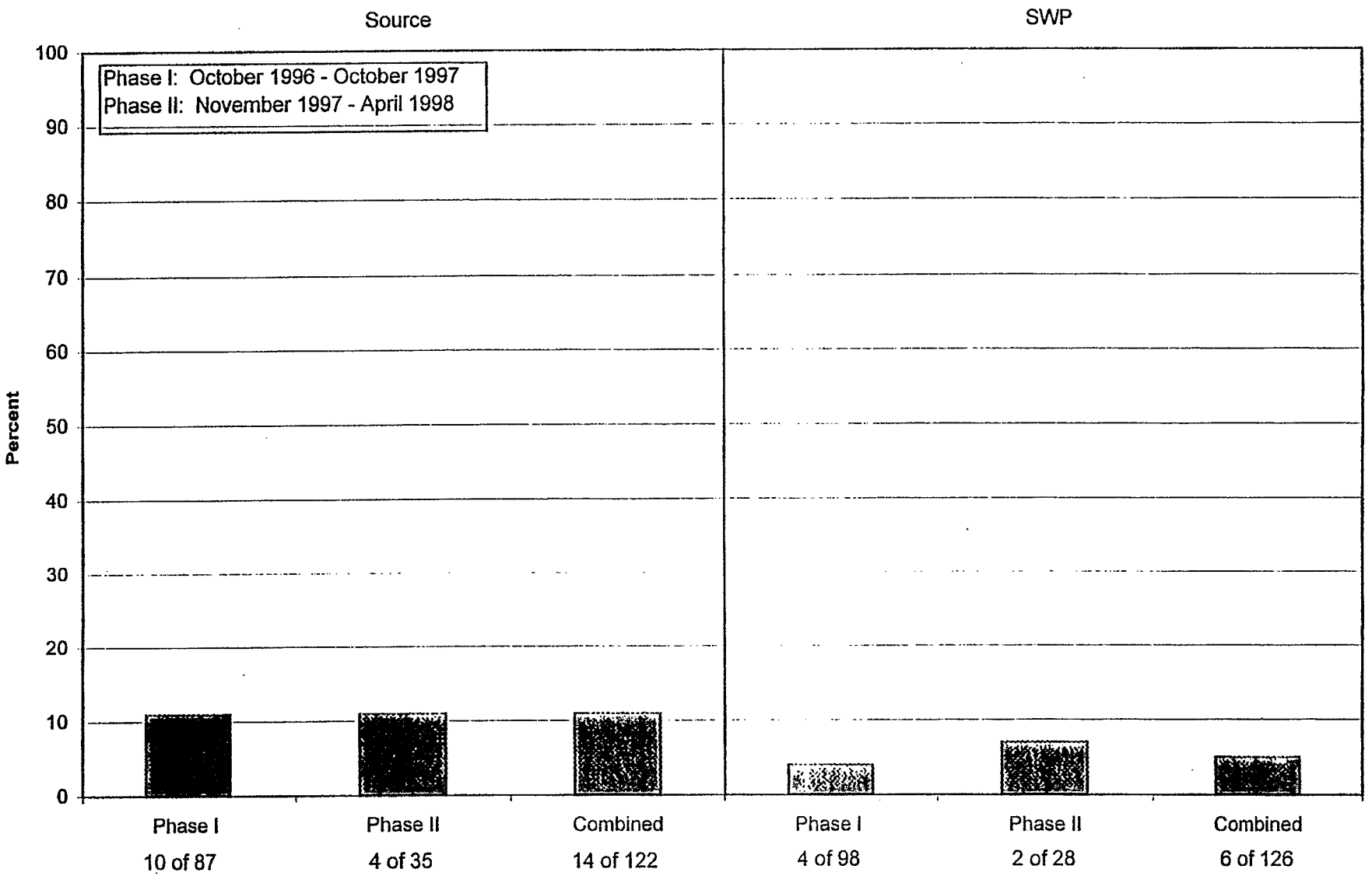
Figure 3-5. *Giardia* Positive Samples - Source Versus SWP



GiardiaCryptoDataforPhase1and2.xls

Fig35

Figure 3-6. *Cryptosporidium* Positive Samples - Source Versus SWP



GiardiaCryptoDataforPhase1and2.xls

Fig36

3-19

Water velocity or flow is not constant in the SWP system and may vary greatly depending on need, which determines pumping and delivery schedules. Settling could occur both in the aqueduct when pumping at the Clifton Court intake is either stopped or reduced, and in the reservoirs as water is discharged from the aqueduct or pipes to calmer waters. Sediment is known to be deposited in the aqueduct when pumping is not occurring and resuspended when pumping resumes, such as in the late spring and summer when water is needed for irrigation purposes (DWR 1996a). Whether or not protozoan cysts are resuspended along with the sediment, and whether they remain viable, has not been determined.

Samples from source waters, the aqueduct, and most event locations were collected from a single point location in the channel or water body and in compliance with the USEPA ICR sampling protocol (except for the grab samples), which is similar to a typical water treatment plant intake arrangement, and at a depth of three feet below the surface and in the main current, where applicable. Samples from reservoirs were collected from the outlet tower when available, which was intended to approximate the water that is actually exported from the reservoir. Whenever possible, reservoir samples were collected at the actual intake for a drinking water treatment plant, such as at MWD's Jensen WTP at Castaic Lake and Mills WTP at Silverwood Lake.

It was assumed that when samples were collected from either an outlet tower or a WTP intake, the water and any organisms it contains have been exposed to whatever fate processes are at work in that particular body of water, and would be representative of the water that is exported. However, water may not always be moving in the direction of the outlet tower or WTP intake in the reservoirs at the time of sampling if water is not being exported, and may not be moving in the aqueduct itself for the same reason. This situation may be more common in reservoirs from which exports are relatively infrequent, such as Lake Perris, and in the aqueduct at certain times of the

year when pumping at the SWP intake at Banks Pumping Plant is either not occurring or is reduced.

This difference between waters within the SWP and source waters did not appear to be related to any change in the performance of the USEPA ICR Protozoan Method related to possible physical or chemical changes in the water as it moves from the river and Delta source through the SWP system, a distance of nearly 600 miles. Changes in method performance related to changes in water quality would be expected to be observed in the split spiked matrix recovery study (see Chapter 2), which used matrix water obtained from five separate locations throughout the study are; such changes in method performance were not apparent.

Protozoan Results, Method Recovery, and Detection Limits

It should not be assumed that because protozoa were not detected by the USEPA ICR Protozoan Method used in this study, that they were not present at sampling locations with low detection frequencies, or at those with none detected. The previously discussed low recovery of both protozoa obtained using the ICR method (see Chapter 2) in this study, particularly for *Cryptosporidium*, and the resulting overall poor precision and accuracy of the method, affects both the quantitative and qualitative aspects of the results obtained with it's use.

The CPMP Study established a goal of attempting to reach a detection limit of 10 cysts or oocysts/100L for each sample collected. Reaching this detection limit goal with 100 percent recovery is very different than reaching it with significantly less than 100 percent recovery. Higher concentrations of cysts or oocysts would have to present to be detected at lower recoveries in order to achieve the same laboratory calculated detection limit of 10 cysts or cysts/100L, which results in a much higher effective detection limit.

The average detection limits for all samples collected for this study was about 17 cysts or oocysts/100L (Table 3-4). As shown in Figures 3-7 and 3-8, the average detection limit for each sampling site was also variable, and was dependant on the physical and chemical properties of the water matrix at each site, of which turbidity is a component. Some sampling sites, such as Vernalis on the San Joaquin River, had significantly higher average detection limits than others, which complicated direct comparisons between sites. Appendix D contains tables displaying detection limits, ranges, and geometric means for the monthly and event samples for all stations.

A frequency histogram (Figure 3-9) for all samples collected for the study shows that the majority of samples were at or near the study detection limit goal of 10 cysts or oocysts per 100L. This frequency histogram further separates the samples into samples collected from sampling sites within the SWP, and those from SWP source waters. Samples from within the SWP system generally had lower average detection limits than those from source waters, probably reflecting processes that occurred as the water traveled through the SWP system, such as particulate settling in SWP reservoirs.

CLOSTRIDIUM perfringins, TOTAL/FECAL COLIFORMS AND E. COLI RESULTS

C. perfringins

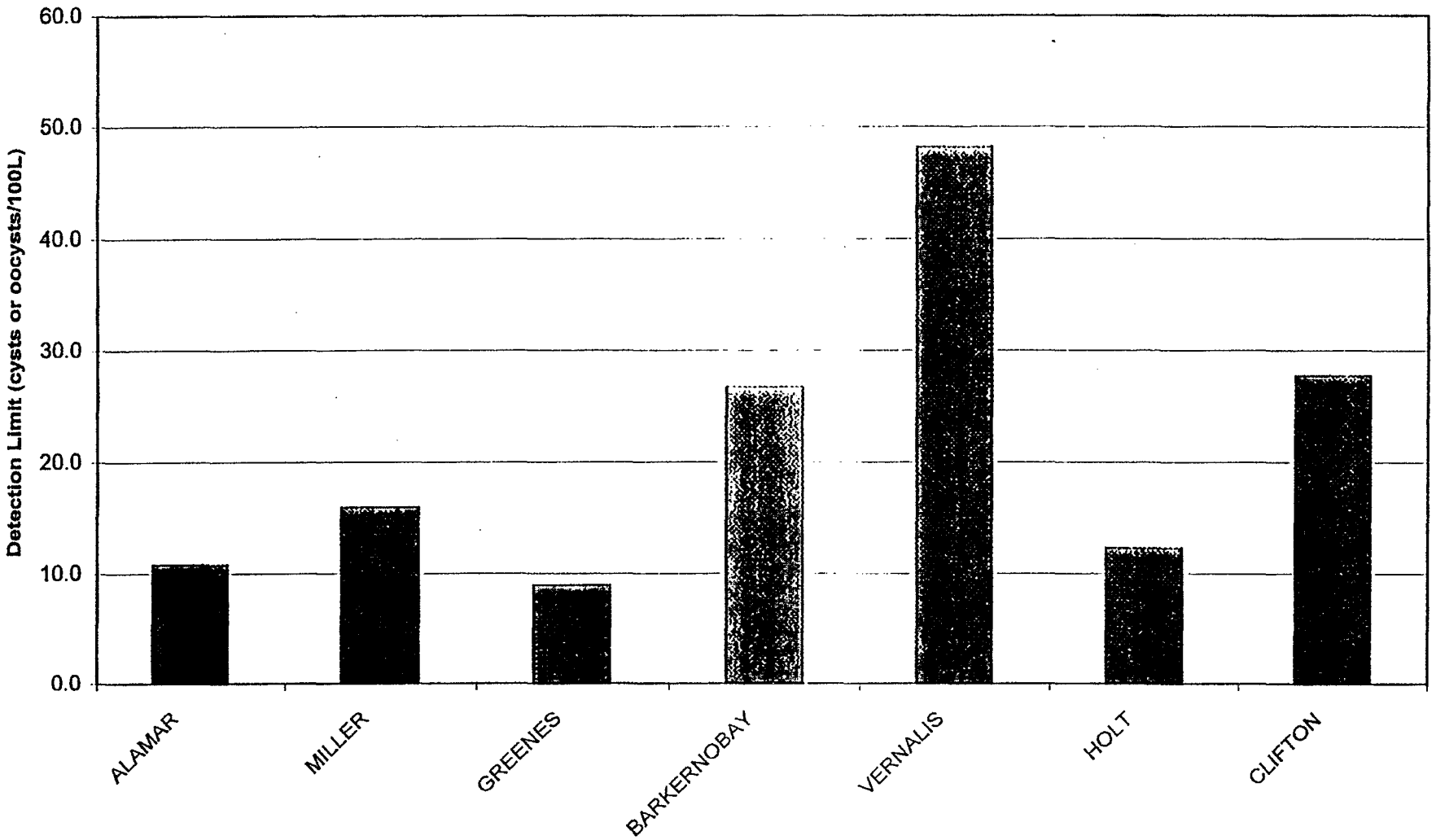
The range of positive *C. perfringins* concentrations was 2 - 400 CFUs/100 mL, with a geometric mean of 25 CFUs/100 mL in monthly samples, and 30 - 800 CFUs/100 mL in event samples, with a geometric mean of 132 CFUs/100 mL (Table 3-5). Detection frequency as the percent of positive samples was 22 percent in monthly samples and 55 percent in event samples.

Table 3-4.

Sample Summary

	Monthly	Event	Combined
Average Sample Volume	103L	99L	102L
Average Detection Limit	11.8	36.6	17.1
Average Number of Slides per Sample	2.8	3.6	3.0
N	195	53	248

Figure 3-7. Average *Giardia* and *Cryptosporidium* Detection Limits for Source Water Sampling Sites



3-24

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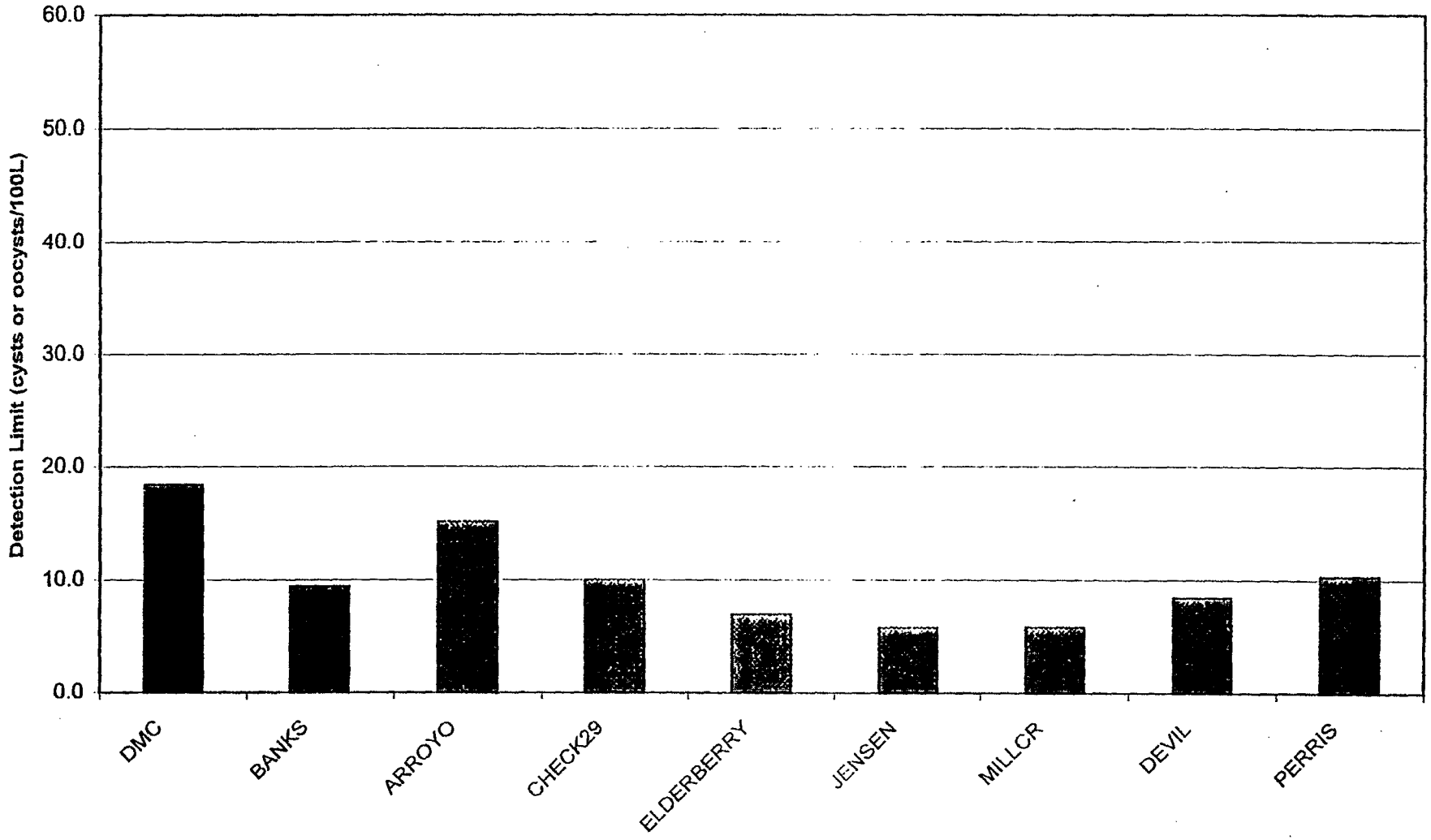
Fig37

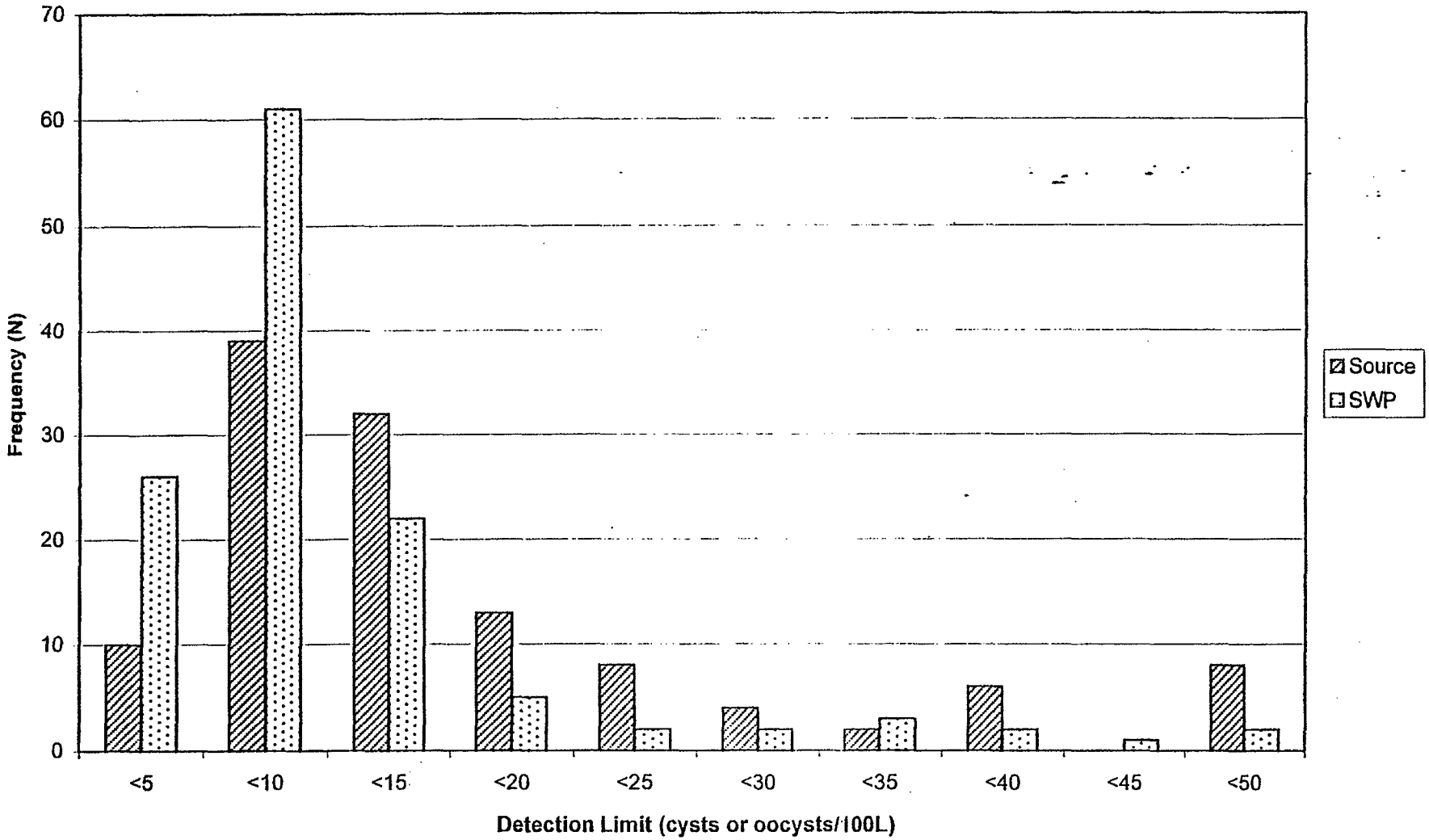
Figure 3-8. Average *Giardia* and *Cryptosporidium* Detection Limits for State Water Project Sampling Sites



RepLmtQuer_Crosstab.xls

Fig38

Figure 3-9. Histogram of *Giardia* and *Cryptosporidium* Detection Limits for Source and SWP Sampling Sites



RepLmtQuer_Crosstab.xls

Fig39

Table 3-5. *C. perfringens* Summary Statistics
(ICR method with units in Colony Forming Units/100mL)

Study		Number Positive	Range	Geometric Mean	Average Detection Limit
CPMP Monthly	Phase I	21% (30 of 143)	2 - 400	24.6	23.3
	Phase II	25% (9 of 36)	20 - 80	28.5	20.0
	Combined	22% (39 of 179)	2 - 400	25.4	22.7
CPMP Event	Phase I	62% (10 of 16)	30 - 800	216.9	23.3
	Phase II	50% (13 of 26)	40 - 200	90.4	20.0
	Combined	55% (23 of 42)	30 - 800	132.2	21.1
CPMP Combined	Phase I	25% (40 of 159)	2 - 800	42.3	23.3
	Phase II	35% (22 of 62)	20 - 200	56.3	20.0
	Combined	28% (62 of 221)	2 - 800	46.9	22.5
Source Combined	Phase I	41% (31 of 76)	2 - 800	55.8	27.1
	Phase II	54% (19 of 35)	20 - 200	62.6	20.0
	Combined	45% (50 of 111)	2 - 800	58.3	25.2
SWP Combined	Phase I	11% (9 of 83)	3 - 300	16.4	21.0
	Phase II	11% (3 of 27)	20 - 60	28.8	20.0
	Combined	11% (12 of 110)	3 - 300	18.9	20.8

As with the protozoans, both detection frequency and concentrations were highest in the Sacramento and San Joaquin river source waters and Delta (45 percent positive) compared to the SWP Aqueduct and reservoirs (11 percent positive). Event sample detection frequency and geometric means were higher than those of the monthly samples, although there were fewer event samples collected relative to monthly samples (Table 3-5). The highest frequency of detection was at the North Bay Aqueduct sampling location at Barker Slough, which also had the highest geometric mean of all monthly sampling locations. The results by station are located in Appendix D.

Total/Fecal coliforms and *E. coli*

The range of fecal coliforms seen in the study was 2-8,000 MPN/100 mL for monthly samples, and 11-22,000 MPN/100 mL for event samples (Table 3-6). For *E. coli* the range of monthly sample results was 2-8,000 MPN/100 mL, and 8-160,000 MPN/100 mL for event samples. As with the *Giardia*, *Cryptosporidium,* and *C. perfringins*, coliform and *E. coli* results were higher at the Sacramento River, San Joaquin River, and the Delta source water sampling locations compared with either aqueduct or reservoir SWP locations (Table 3-7). Total/fecal coliforms and *E. coli* were present 96 to 100 percent of the time at source water sampling sites, and between 70 and 87 percent of the time at sampling locations within the SWP system (Table 3-7).

The highest concentration of fecal coliforms and *E. coli* in monthly samples (8,000 MPN/ 100mL) was determined at the Alamar sampling location, the most northern and upstream site on the Sacramento River (Appendix D). The highest event sample concentration for *E. coli* was at the Greene's Landing storm sampling site (16,000 MPN/ 100 mL), while the highest fecal coliform result was at the Clifton Court site (22,000 MPN/100 mL). Where the protozoan, *C. perfringins*, and total coliform

Table 3-6. Total and Fecal Coliform, and *E. coli* Summary Statistics
(units in Most Probable Number/100mL)

		Total Coliform				Fecal Coliform			*E. coli*		
		N	No. Pos.	Range	Geo. Mean	% Pos.	Range	Geo. Mean	% Pos.	Range	Geo. Mean
Study Summary											
Monthly	Phase I		136 of 148	2 - 50,000	118	122 of 152	2 - 8,000	37.7	116 of 151	2 - 8,000	29.8
	Phase II		35 of 36	4 - 1,700	117	34 of 36	4 - 1,700	52.2	34 of 36	2 - 1,700	48.9
	Combined		171 of 184	2 - 50,000	118	156 of 188	2 - 8,000	40.5	150 of 187	2 - 8,000	33.3
Event	Phase I		26 of 28	13 - 160,000	1,400	25 of 28	11 - 22,000	279	19 of 22	11 - 6,000	176
	Phase II		26 of 26	11 - 30,000	1,133	26 of 26	4 - 16,000	374	26 of 26	8 - 16,000	354
	Combined		52 of 54	11 - 160,000	1,260	51 of 54	4 - 22,000	324	45 of 48	8 - 16,000	264
Combined	Phase I		162 of 176	2 - 160,000	176	147 of 180	2 - 22,000	53.0	135 of 173	2 - 8,000	38.2
	Phase II		61 of 62	4 - 30,000	308	60 of 62	4 - 16,000	122	60 of 62	2 - 16,000	115
	Combined		223 of 238	2 - 160,000	205	207 of 242	2 - 22,000	67.6	195 of 235	2 - 16,000	53.7

Table 3-7. Total and Fecal Coliform, and *E. coli* Summary Statistics
(units in Most Probable Number/100mL)

		Total Coliform			Fecal Coliform			*E. coli*		
	N	No. Pos.	Range	Geo. Mean	% Pos.	Range	Geo. Mean	% Pos.	Range	Geo. Mean
Source Summary										
Monthly	Phase I	69 of 69	2 - 50,000	373	69 of 70	2 - 8,000	69.4	65 of 70	2 - 8,000	51.9
	Phase II	19 of 19	50 - 1,700	237	19 of 19	4 - 1,700	91.9	19 of 19	4 - 1,700	95.0
	Combined	88 of 88	2 - 50,000	338	88 of 89	2 - 8,000	73.7	84 of 89	2 - 8,000	59.5
Event	Phase I	16 of 16	210 - 160,000	2,529	16 of 16	14 - 22,000	360	14 of 14	14 - 6,000	238
	Phase II	16 of 16	130 - 30,000	2,430	16 of 16	23 - 16,000	846	16 of 16	23 - 16,000	785
	Combined	32 of 32	130 - 160,000	2,479	32 of 32	14 - 22,000	552	30 of 30	14 - 16,000	450
Combined	Phase I	85 of 85	2 - 160,000	535	85 of 86	2 - 22,000	94.6	79 of 84	2 - 8,000	68.0
	Phase II	35 of 35	50 - 30,000	686	35 of 35	4 - 16,000	253	35 of 35	4 - 16,000	249
	Combined	120 of 120	2 - 160,000	575	120 of 121	2 - 22,000	126	114 of 119	2 - 16,000	101
SWP Summary										
Monthly	Phase I	67 of 79	2 - 1,600	36.2	53 of 82	2 - 500	17.0	51 of 81	2 - 300	14.7
	Phase II	16 of 17	4 - 800	50.7	15 of 17	4 - 280	25.5	15 of 17	2 - 220	21.1
	Combined	83 of 96	2 - 1,600	38.7	68 of 99	2 - 500	18.6	66 of 98	2 - 300	15.9
Event	Phase I	10 of 12	13 - 3,000	543	9 of 12	11 - 1,600	177	5 of 8	11 - 800	75.3
	Phase II	10 of 10	11 - 9,000	334	10 of 10	4 - 2,400	101	10 of 10	8 - 2,400	99.1
	Combined	20 of 22	11 - 9,000	426	19 of 22	4 - 2,400	132	15 of 18	8 - 2,400	90.4
Combined	Phase I	77 of 91	2 - 3,000	51.5	62 of 94	2 - 1,600	23.9	53 of 89	2 - 800	17.0
	Phase II	26 of 28	4 - 9,000	105	25 of 28	4 - 2,400	44.3	25 of 28	2 - 2,400	39.2
	Combined	103 of 118	2 - 9,000	61.6	87 of 121	2 - 2,400	28.6	81 of 116	2 - 2,400	22.0

concentrations were highest in the event samples, the highest fecal coliform and *E. coli* concentrations were found in the monthly sample group.

FLOOD EVENT OF JANUARY 1997

The provisions made in the study design for contingency samples were used for the January 1997 floods in order to gain information about the pathogen levels of flood waters. Selected storm event sampling locations were sampled during the week of January 6-10, 1997, with several additional locations added to sample flood waters in specific areas (see Table 1-2 for flood event locations). Each flood event location was sampled for all organisms included in the CPMP Study, with the results displayed in Table 3-8. These flood samples were included in the overall summary statistics for the event samples, but have been sorted as a subset (a relatively small subset of N=10) of this larger group in order to see how they compare to both the complete monthly and event groups.

The flood samples as a group had the highest geometric mean levels for *Cryptosporidium*, total/fecal coliforms, *E. coli* , and *C. perfringins* when compared with either the monthly or event sample group results for all organisms. Only for *Giardia* was the event sample group geometric mean higher than in the flood event group. Detection frequency, as percent of positive samples, was higher for all organisms/organism classes in the flood event group than for either the monthly or event sample group. The flood group *Giardia* detection frequency was 70 percent positive, with *Cryptosporidium* at 40 percent positive.

CORRELATION ANALYSIS

The search for a surrogate organism or parameter which could be used as an indicator of the presence and, perhaps, even approximate concentrations of *Giardia*

Table 3-8.

Flood Event Sample Statistics

(N = 10)

	Giardia	*Cryptosporidium*	Total Coliform	Fecal Coliform	*E. coli*	*C. perfringins*
Positive Samples	70%	40%	100%	90%	90%	80%
	Protozoa/100L		**MPN/100mL**			**CFU/100mL**
Geometric Mean	58.3	40.2	1492	369.5	248.7	280.6
Range	10.05 - 129.8	10.4 - 200	13 - 28,000	50 - 22,000	40 - 6,000	100 - 800
Average Detection Limit	58.3	40.2				
Detection Limit Range	8.9 - 200					

and *Cryptosporidium* has been an area of continued interest. This interest is directly related to the poor performance characteristics and cost of the currently available analytical methods, which includes the USEPA ICR Protozoan Method. There has generally been little success in determining consistent relationships between the occurrence of other organisms and either *Giardia* or *Cryptosporidium* that could be applied to the general case (all waters), although there are examples of better relationships in specific cases (Butler and Mayfield 1996; Jakubowski and others 1996; and Payment and Franco 1993).

The protozoa share several characteristics which make finding a surrogate indicator difficult, such as having both human and non-human hosts and a long survival time in the environment. In addition, surrogate indicator organisms or parameters should be present when the protozoa are present, and in numbers directly related to

protozoan numbers. Even if no such surrogate indicator has yet been identified, currently available surrogate indicator organisms or parameters for *Giardia* and *Cryptosporidium* do appear to have some utility in defining conditions or patterns of contamination in a watershed or water body which may indicate the potential for the protozoa to be present. Examples of such indicators include turbidity, particle counting, microscopic particulate analysis, coliform and heterotrophic plate counts, *C. perfringins* and aerobic spores, and coliphages (Jakubowski and others 1996).

General Correlation

A correlation analysis measures the relationship between two data sets. It is used to determine whether two ranges of data (e.g., total coliforms vs. *E. coli*) move together, with large values of one set associated with large values of the other (a positive correlation). There can also be a negative correlation with small values of one set associated with large values of the other. When values in both sets of data are unrelated, a correlation coefficient near zero is obtained, indicating little or no correlation between the data sets.

Data collected during the first year of the study from all sample collection sites with analytical laboratory results for total and fecal coliforms, *E. coli*, *C. perfringins*, *Giardia*, *Cryptosporidium*, plus field turbidity measurements were compared. Sites were sampled from one to 14 times with the major sites sampled monthly in addition to storm events. The pool of data – including zeros for non-detects and no results – consists of 1,274 entries for the seven parameters.

Results of the correlation analysis are reported in Table 3-9. It is apparent that, overall, the data are not well correlated. Only the relationship between fecal coliforms and *E. coli* exhibits a correlation coefficient greater than 80 percent and only two more

Table 3-9. Correlation of Data from the Following Sampling Stations

ALAMAR	MILLER	GREENES	MOKELUMNE	SHAG	JENSEN	CHECK29
DEVIL	MILLCR	PIRU	BANKS	HOLT	DMC	
CLIFTON	VERNALIS	PERRIS	ELDERBERRY	ARROYO	BARKERNOBAY	

CORRELATION

	Total Coliform	Fecal Coliform	*E. coli*	*C. perfringens*	*Giardia*	*Cryptosporidium*	Field Turbidity
Total Coliform	1						
Fecal Coliform	0.258	1					
E. coli	0.223	0.823	1				
C. perfringens	0.174	0.629	0.426	1			
Giardia	0.118	0.458	0.376	0.511	1		
Cryptosporidium	0.005	0.019	0.011	0.102	0.066	1	
Field Turbidity	0.052	0.196	0.145	0.292	0.197	0.147	1

sets (fecal vs. *C. perfringins* and *C. perfringins* vs. *Giardia*) exhibit coefficients greater than 50 percent.

Given the analytical uncertainties in making biological measurements and the resultant questionable analytical results, and particularly when combined with the use of the USEPA ICR Protozoan Method, the biological data were transformed by calculating the percent positive results found at the 15 sampling sites--sampled three or more times --and the correlation analysis performed again. Since percent positive calculations are not appropriate for field turbidity measurements, this parameter was dropped from the correlation analysis.

The results with this transformed data are in Table 3-10, and show some improvement in the correlation coefficients; eight of the correlation coefficients are greater than 50 percent. However, there is still only one coefficient (total coliforms vs. *E. coli*) over 80 percent.

Site-specific Correlation

It was recognized that all sampling sites were not equivalent in terms of the potential for finding biological contamination, so correlation analyses were run for those individual sites where adequate sample data existed (Table 3-11). In this table data from one set of sites (MILLCR, PIRU, SHAG, and MOKELUMNE) were combined, and indicate that there are sample collection sites with a number of correlations demonstrated.

The largest number of correlations (15) with a coefficient ≥ 0.8 occurred at Clifton Court, sampled only three times, during and following a storm event. Most other sites sampled from nine to 16 times over the year generally exhibit two to four correlations; three sites (ALAMAR, MILLER, and HOLT) exhibit seven, seven, and

Table 3-10. Overall Correlation with Raw Data Values Changed to Reflect Percent Positive at Each Site (with Three or More Datum) Since Single Value per Site, No Correlation with Field Turbidity Possible

	CORRELATION					
	Total Coliform	Fecal Coliform	*E. coli*	*C. perfringens*	*Giardia*	*Cryptosporidium*
Total Coliform	1					
Fecal Coliform	0.732	1				
E. coli	0.912	0.748	1			
C. perfringens	0.504	0.397	0.570	1		
Giardia	0.591	0.542	0.611	0.486	1	
Cryptosporidium	0.393	0.149	0.412	0.008	0.426	1

Table 3-11. Correlations Greater Than 0.8

Sampling Site	Total Coliforms						Fecal Coliforms					*E. coli*				*C. perfringens*			*Giardia*		*Crypto.*
	T/F	T/E	T/C	T/G	T/Cry	T/Tu	F/E	F/C	F/G	F/Cry	F/Tu	E/C	E/G	E/Cry	E/Tu	C/G	C/Cry	C/Tu	G/Cry	G/Tu	Cry/Tu
CLIFTON	X	X	X	X		X	X	X	X		X	X	X		X	X		X		X	
HOLT							X	X			X	X			X			X			
ALAMAR	X	X					X									X	X	X			X
MILLER	X	X			X		X			X				X				X			
DMC	X	X					X											X			
CHECK29	X	X					X														
DEVIL	X	X					X														
M-C,P,Sh, Mok	X	X					X				X				X		X				
PERRIS							X	X				X									
JENSEN							X	X				X									
ELDERBERRY							X														
GREENES							X				X				X						
BARKERNOBAY						X											X				
ARROYO					X					X											
BANKS	X				X																
VERNALIS												X		X			X				
OVERALL							X														

Legend

MILLCR =	M-C	T = Total Coliforms	C = *C. perfringens*
PIRU, SHAG =	P, Sh	F = Fecal Coliforms	G = *Gardia*
MOKELUMNE =	Mok	E = *E. coli*	Cry = *Cryptosporidium*
		Tu = Turbidity(Field)	

six correlations, respectively. The most frequent correlation (12 times) is between fecal coliforms and *E. coli*. The next two (eight and seven times) are between total coliforms and fecal coliforms, and between total coliforms and *E. coli*.

Giardia never correlated with *Cryptosporidium* in these data, and only once with total coliforms, fecal coliforms, turbidity, and *E. coli*, and only twice with *C. perfringins*. Similarly, turbidity correlated only once with total coliforms, *Giardia*, and *Cryptosporidium*, but four times with fecal coliforms and *E. coli*, and five times with *C. perfringins*. Total coliforms correlated only once with *C. perfringins*.

It appears from these results that any conclusions regarding how parameters might correlate are site specific. Upon further analysis, they may also be found to be seasonally specific or episodic. As previously discussed, this finding is consistent with other efforts to find surrogates for *Giardia* and *Cryptosporidium*.

Graphical Groupings

In addition to calculating the correlation between pairs of environmental parameters at a sampling site, the results can be graphically presented. In Figures 3-10 through 3-14 are graphs of the various analytical and field results plotted versus sampling dates for selected sites. These graphs are plotted with double y-axes so that total and fecal coliforms, *E. coli*, and *C. perfringins* have a separate scaling factor from *Giardia*, *Cryptosporidium*, and field turbidity. This has the effect of increasing the height of the latter data points on the graph so that up-and-down trends can be seen better. Figures 3-12 and 3-14 represent sites with seven parameter correlations ≥ 0.8. There are six correlations found at HOLT (Figure 3-12), but only three at GREENES (Figure 3-13), and two at BARKERNOBAY (Figure 3-14).

Figure 3-10. Sacramento River at Miller Park Correlations Greater Than 0.8.

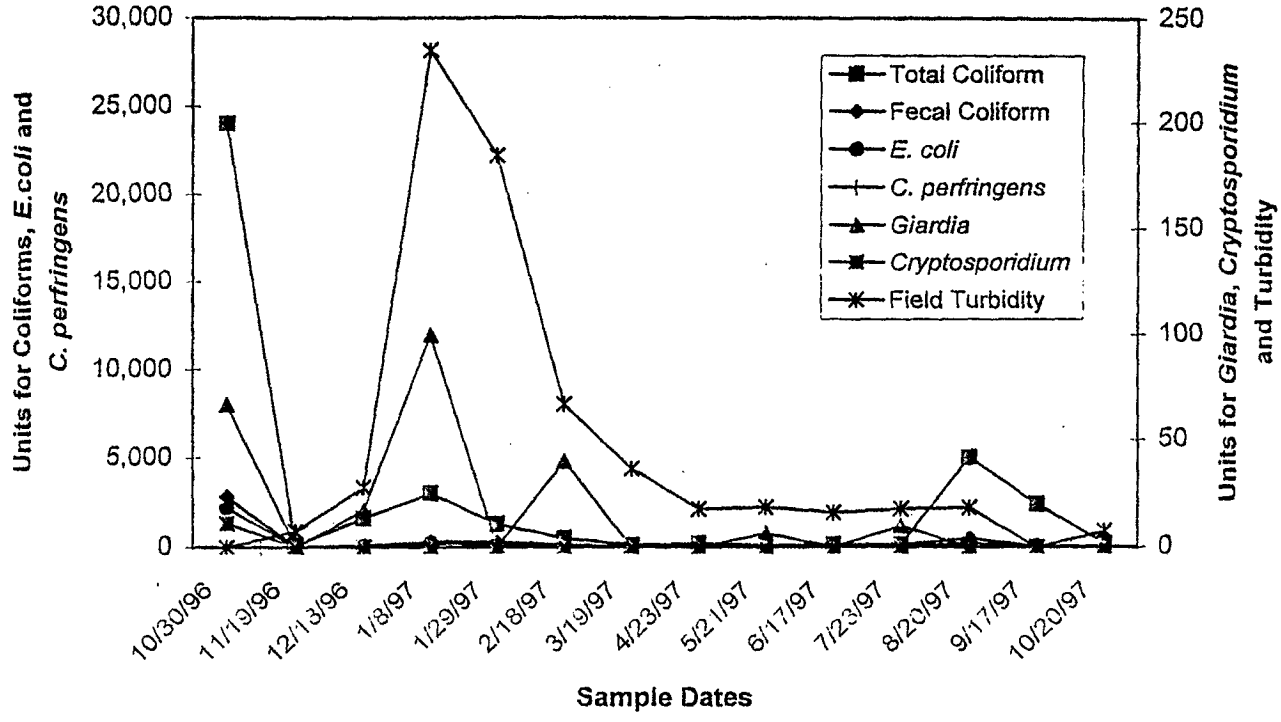


Figure 3-11. Sacramento River at Alamar Marina Correlations Greater Than 0.8.

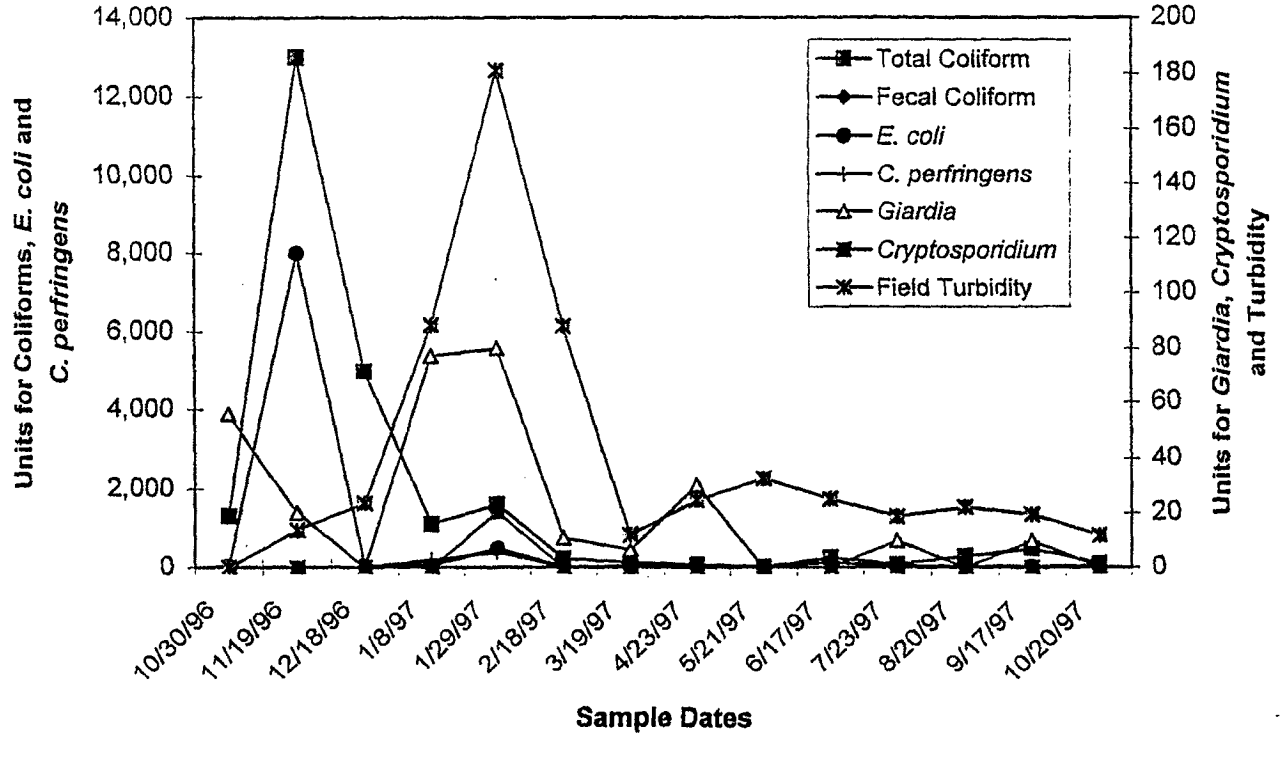


Figure 3-12. San Joaquin River at Holt Road Correlations Greater Than 0.8.

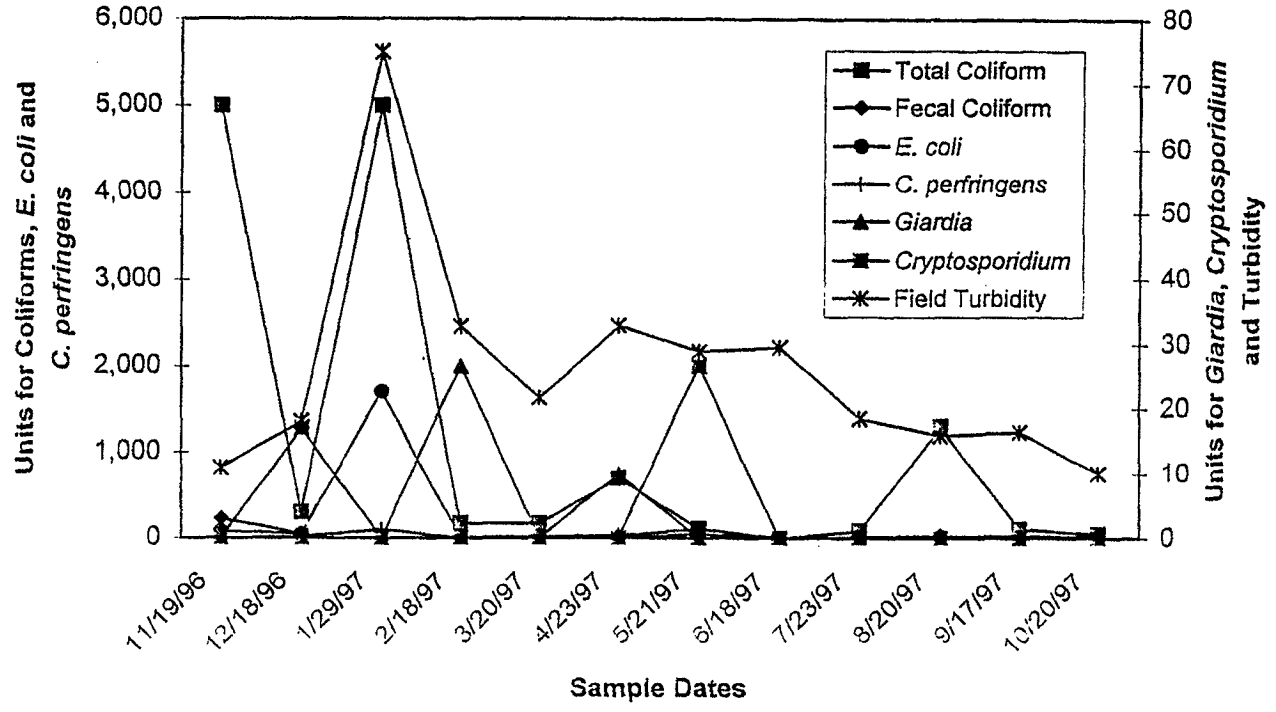


Figure 3-13. Sacramento River at Greenes Landing Correlations Greater Than 0.8.

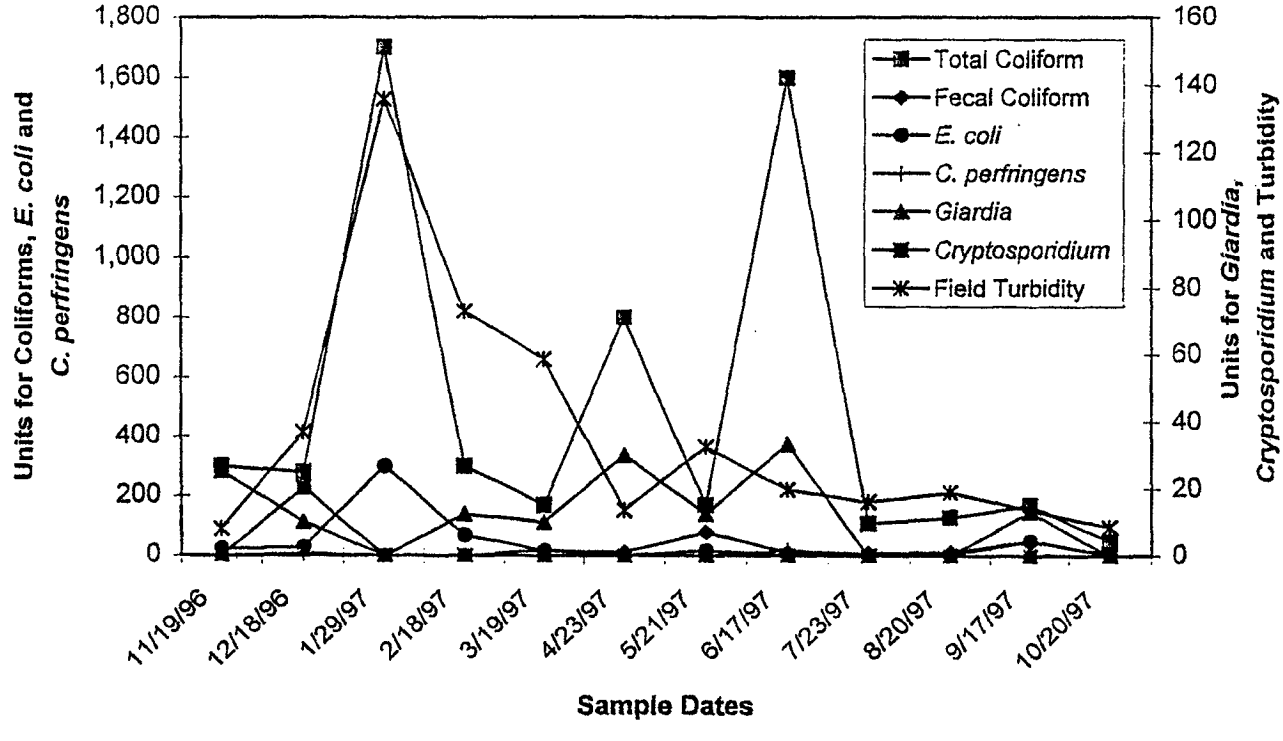
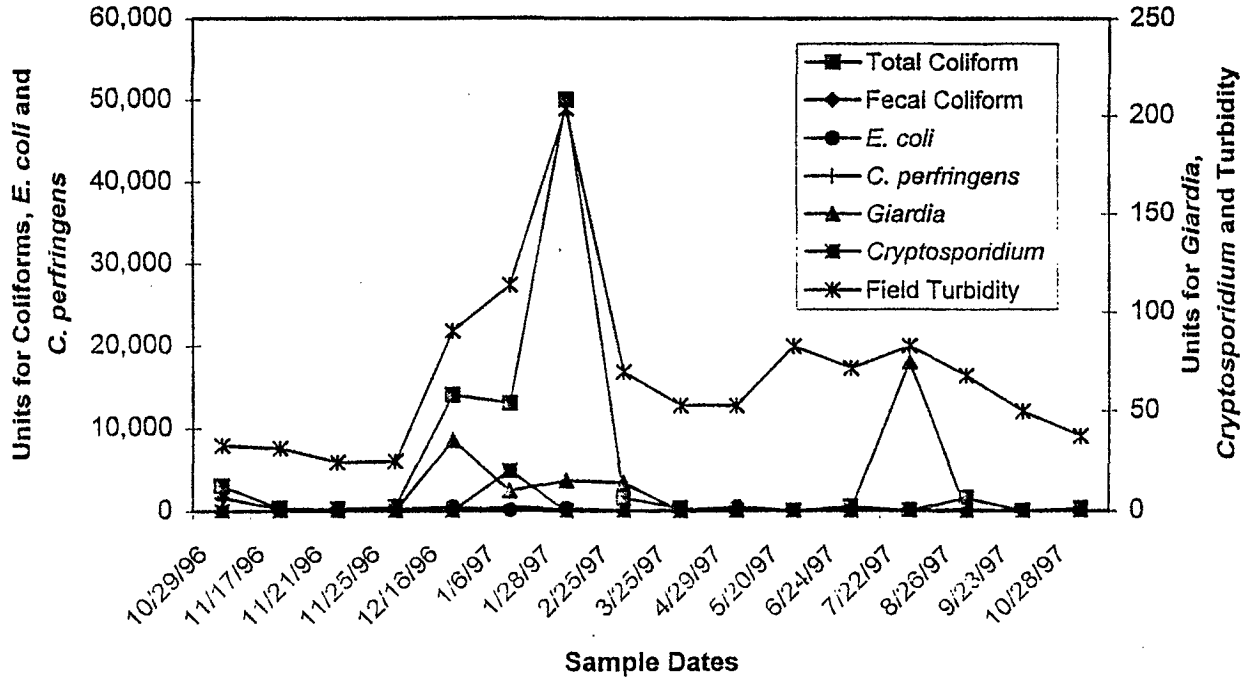


Figure 3-14. North Bay Aqueduct at Barker Slough Pumping Plant Correlations Greater Than 0.8.



The correlation results for each of these sites have been reproduced so that the correlated parameters can be compared with the data trends portrayed on the respective graphs (Table 3-12). However, it is still difficult to visually observe most of the positive correlations.

Finally, to see whether the data from storm or flood events might show a better correlation, they were selected and the correlation between parameters determined. The results are shown in Table 3-13, and indicate that the extent of correlation between parameters did not improve. In general, the correlation is less significant than when all sampled sites were considered (Table 3-9). What impact the low and variable recovery of protozoa by the analytical method used had on the correlation calculations is unknown. However, both quantitative and qualitative (as percent of positives) aspects of the correlation analysis may be to some extent affected by method performance.

Table 3-12. Correlation Results for Selected Sampling Stations

MILLER	CORRELATION						
	Total Coliform	Fecal Coliform	*E. coli*	*C. perfringens*	*Giardia*	*Cryptosporidium*	Field Turbidity
Total Coliform	1						
Fecal Coliform	0.991	1					
E. coli	0.988	0.996	1				
C. perfringens	-0.053	-0.016	-0.029	1			
Giardia	0.509	0.506	0.530	0.307	1		
Cryptosporidium	0.973	0.981	0.991	-0.111	0.462	1	
Field Turbidity	-0.117	-0.089	-0.091	0.912	0.546	-0.187	1

ALAMAR	CORRELATION						
	Total Coliform	Fecal Coliform	*E. coli*	*C. perfringens*	*Giardia*	*Cryptosporidium*	Field Turbidity
Total Coliform	1						
Fecal Coliform	0.930	1					
E. coli	0.931	1.000	1				
C. perfringens	-0.041	-0.063	-0.064	1			
Giardia	0.035	0.020	0.015	0.788	1		
Cryptosporidium	-0.008	-0.020	-0.019	0.888	0.589	1	
Field Turbidity	-0.099	-0.109	-0.110	0.905	0.651	0.840	1

HOLT	CORRELATION						
	Total Coliform	Fecal Coliform	*E. coli*	*C. perfringens*	*Giardia*	*Cryptosporidium*	Field Turbidity
Total Coliform	1						
Fecal Coliform	0.741	1					
E. coli	0.685	0.996	1				
C. perfringens	0.590	0.901	0.909	1			
Giardia	-0.172	-0.149	-0.136	-0.032	1		
Cryptosporidium	-0.219	-0.095	-0.108	-0.094	-0.174	1	
Field Turbidity	0.445	0.861	0.879	0.882	0.165	-0.032	1

Table 3-12. Correlation Results for Selected Sampling Stations *(Continued)*

GREENES	CORRELATION						
	Total Coliform	Fecal Coliform	*E. coli*	*C. perfringens*	*Giardia*	*Cryptosporidium*	Field Turbidity
Total Coliform	1						
Fecal Coliform	0.587	1					
E. coli	0.598	0.977	1				
C. perfringens	0.298	-0.231	-0.199	1			
Giardia	0.355	-0.299	-0.314	0.359	1		
Cryptosporidium	-0.110	-0.084	-0.051	0.125	-0.055	1	
Field Turbidity	0.508	0.883	0.894	0.037	-0.330	0.006	1

BARKERNOBAY	CORRELATION						
	Total Coliform	Fecal Coliform	*E. coli*	*C. perfringens*	*Giardia*	*Cryptosporidium*	Field Turbidity
Total Coliform	1						
Fecal Coliform	0.068	1					
E. coli	0.318	0.127	1				
C. perfringens	0.501	-0.134	0.000	1			
Giardia	0.162	-0.130	0.004	0.041	1		
Cryptosporidium	0.161	-0.108	-0.054	0.922	0.009	1	
Field Turbidity	0.890	-0.180	0.166	0.591	0.335	0.274	1

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Table 3-13. Correlation of Data from Storm and Flood Events

	Total Coliform	Fecal Coliform	*E. coli*	*C. perfringens*	*Giardia*	*Cryptosporidium*	Field Turbidity
Total Coliform	1						
Fecal Coliform	0.196	1					
E. coli	0.145	0.820	1				
C. perfringens	0.073	0.599	0.306	1			
Giardia	0.046	0.493	0.338	0.509	1		
Cryptosporidium	-0.070	-0.052	-0.043	-0.022	-0.037	1	
Field Turbidity	-0.015	0.121	0.121	0.120	0.044	0.054	1

Chapter 4

SUMMARY AND CONCLUSIONS

SUMMARY

Methods

The USEPA's ICR methods for *Giardia* and *Cryptosporidium*, and for *C. perfringins* were used for this study. A MDL goal of 10 cysts or oocysts/100L (total IFA count) for the protozoa was specified for this project. Total, fecal coliforms, and E. coli were analyzed using the 5-tube, 5-dilution MPN method from *Standard Methods for the Examination of Water and Wastewater*, 19th Edition.

The USEPA ICR Protozoan Method has several performance characteristics which should be considered when interpreting results obtained using it. As summarized by the USEPA, the ICR Protozoan Method is "...difficult to run, has poor recovery, and does not have a high level of precision." The ICR Protozoan Method's performance is subject to limitations at all steps in the procedure.

Split Spiked Matrix Recovery Study

QA/QC were provided as required by the analytical methods, in compliance with the ICR where applicable, and in accordance with existing DWR/DPLA QA/QC

DWR/DPLA QA/QC protocols. In addition, a split spiked matrix study was conducted using matrix water obtained from five locations distributed throughout the project area. This recovery study was also intended to demonstrate that the USEPA ICR method's performance was consistent throughout the project area, which covers a distance of approximately 600 miles.

The average recovery of spiked *Giardia* cysts was 2.53 percent, and 0.35 percent for spiked *Cryptosporidium* oocysts. The low recoveries for both protozoa, the large standard deviations, along with 50 percent non-detects for *Cryptosporidium* in spiked samples is indicative of the performance concerns related to the use of this method, and of the difficulties in interpreting the results obtained using it. According to the USEPA, experience using the ICR Protozoan Method has shown that it underestimates both the detection frequency and levels of both *Giardia* and *Cryptosporidium*.

A detection limit goal of 10 cysts or oocysts per 100L was specified and achieved for both the split matrix spike samples and approached for most samples throughout the CPMP Study. The ability to achieve this detection limit apparently had no effect on the ICR method's ability to detect either approximately 1,100 *Cryptosporidium* oocysts or 900 *Giardia* cysts spiked per sample (100 liters), with the low recoveries for both protozoa a factor. The results also demonstrated that method performance is generally consistent with all water matrices obtained from within the project area.

Percent Positive, Geometric Mean, and Range of CPMP Data

All *Giardia* and *Cryptosporidium* results discussed in this report are reported as total IFA counts, which represents a conservative use of the data. The range of positive monthly CPMP *Giardia* results was 2.4 to 92.3 cysts/100L, with a geometric mean of 16.4 cysts/100L. Both the range (10 - 140 cysts/100L) and geometric mean

(40.9 cysts/100L) of the CPMP storm event samples were higher than the CPMP monthly samples. The percent of positive *Giardia* samples for the CPMP storm event samples (34 percent) was higher than that of the monthly samples (24 percent), with the MWD Study (13 percent) somewhat lower.

The range of positive monthly CPMP *Cryptosporidium* results was 9.0 - 26.7 oocysts/100L, with a geometric mean of 17.2 oocysts/100L. As with *Giardia*, both the range (4.4 - 200 oocysts/100L) and geometric mean (29.6 oocysts/100L) of the *Cryptosporidium* CPMP event samples were higher than that of the CPMP monthly samples. The *Cryptosporidium* percent positive for the CPMP storm event samples (25 percent) was higher than that of the monthly samples (4 percent), with the MWD Study (35 percent) somewhat higher than either the CPMP event or monthly samples.

The highest detection frequencies for Giardia are found in the SWP's Sacramento and San Joaquin River source waters for the SWP. The highest detection frequency (71 percent positive) for Giardia was at the Greenes Landing sampling site on the Sacramento River below the cities of Sacramento and West Sacramento. Giardia was not detected at all, and *Cryptosporidium* only once in a storm event sample (Clifton Court) at Banks Pumping Plant, the intake for the SWP system.

Like Giardia, *Cryptosporidium* detection was more frequent in the Sacramento and San Joaquin River source waters of the SWP compared with the sampling locations within the SWP system itself, including the aqueduct and reservoirs. The fate processes affecting both the presence and concentrations of these organisms are not well defined. This difference does not appear to be related to any change in the performance of the USEPA ICR Protozoan Method caused by possible physical or chemical changes in the water as it moves from the source through the SWP system, a distance of nearly 600 miles.

For stations where both Giardia and *Cryptosporidium* were detected, the percent of samples positive for Giardia was generally much higher than for *Cryptosporidium*, a result which may be related to the lower recovery of *Cryptosporidium* (0.35 percent) relative to *Giardia* (2.53 percent) using the ICR Protozoan Method in the CPMP Study. The majority of results from samples collected from sampling locations either in the California Aqueduct or in the SWP reservoirs were below the detection limit for both protozoa. Giardia was detected in 50 percent of the source water samples and in only 2 percent of the SWP samples. *Cryptosporidium* was present in 11 percent of the source water samples and in 5 percent of the SWP samples.

The detection frequency, geometric mean, and range of positive results for both protozoans were greater in the storm and flood event samples collected in the wet season compared with the monthly samples, which includes both wet and dry season results. Both protozoa were detected more frequently in the wet season samples relative to the dry season samples, with Giardia wet season/dry season percent positive samples being 31 percent/16 percent, and *Cryptosporidium* being 11 percent/2 percent.

The range of positive *C. perfringins* concentrations in monthly samples was 2 - 400 CFUs/100 mL, with a geometric mean of 25 CFUs/100 mL, and was 30 - 800 CFUs/100 mL in event samples, with a geometric mean of 217 CFUs/100 mL. Detection frequency as the percent of positive samples was 22 percent in monthly samples and 55 percent in event samples. The highest frequency of detection was at the Barker Slough sampling location at the intake to the North Bay Aqueduct, which also had the highest geometric mean of all monthly sampling locations.

As with the protozoans, *C. perfringins*, and total/fecal coliforms and *E. coli* detection frequency and concentrations were highest in the Sacramento River, San Joaquin River, and in the Delta compared with the SWP Aqueduct and reservoirs. Storm and flood event sample detection frequency and geometric means were also

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higher than those of the monthly samples for these organisms. Where the protozoan, *C. perfringins*, and total coliform concentrations were highest in the event samples, the highest fecal coliform and *E. coli* concentrations were found in the monthly sample group.

Additional samples were collected during the January 1997 floods in order to gain information about the pathogen levels of flood waters. Selected storm event sampling locations were sampled during the week of January 6-10, 1997, with several additional locations added to sample flood waters in specific areas. The flood samples as a group had the highest geometric mean for *Cryptosporidium*, total/fecal coliforms, *E. coli* , and *C. perfringins* when compared with either the monthly or event sample group results for all organisms.

Only for *Giardia* was the event sample group geometric mean higher than in the flood event group. Detection frequency, as percent of positive samples, was higher for all organisms/organism classes in the flood event group than for either the monthly or event sample groups. The flood group *Giardia* detection frequency was 70 percent positive samples, with *Cryptosporidium* at 40 percent positive.

Correlation Analysis

Correlation analyses were conducted on the first 12 months of data to determine if the organism and organism classes or turbidity were correlated with each other. The results of the correlation analysis indicate that the data are not well correlated. Only the relationship between fecal coliforms and *E. coli* exhibited a correlation coefficient greater than 80 percent and only two more sets (fecal versus *C. perfringins* and *C. perfringins* versus Giardia) exhibited coefficients greater than 50 percent.

Correlation analyses were also run for those individual sites where adequate sample data existed. *Giardia* never correlated with *Cryptosporidium* in these data groups, and only once with total coliforms, fecal coliforms, turbidity, and *E. coli*, and only twice with *C. perfringins*. It appears from these results that any conclusions regarding how these parameters might correlate are likely to be site specific, and upon further analysis they may also be found to be seasonally specific or episodic. This finding is consistent with other efforts to find surrogates for *Giardia* and *Cryptosporidium*.

CONCLUSIONS

Analytical

- The average recoveries of *Giardia* and *Cryptosporidium* from split spiked matrix samples was 2.53 percent and 0.35 percent, respectively. The USEPA ICR Protozoan Method demonstrated poor recovery, accuracy, and precision in the CPMP Study. The detection frequency and concentrations of both protozoa are likely higher, perhaps significantly higher, than the analytical results indicate.

- Due in part to the low recovery of *Giardia* and *Cryptosporidium*, the detection limit calculated for an ICR protozoan analytical result does not reflect the actual detection limit, which is most likely higher. If the detection limit goal of 10 cysts/oocysts per 100 liters had not been set, even fewer detections would have been observed.

Results

- The range, geometric mean, and percent positive samples of the CPMP event samples were higher compared with the monthly samples. Storm and flood

waters contained higher concentrations of protozoa more frequently than "average" waters.

- When wet season sample results were compared with dry season results, both protozoa were detected more frequently and at higher concentrations in the wet season compared to the dry season, and *Giardia* more frequently than *Cryptosporidium*.

- *Giardia* and *Cryptosporidium*, *C. perfringins*, and total/fecal coliforms and *E. coli* detection frequency and concentrations were highest in the Sacramento and San Joaquin rivers, and in the Delta compared with the SWP Aqueduct and reservoirs. This difference did appear to be related to any change in the performance of the USEPA ICR Protozoan Method caused by possible physical or chemical changes in the water as it moves from the source through the SWP system, a distance of nearly 600 miles.

- *Cryptosporidium* was detected less frequently and at lower concentrations compared to *Giardia* in the CPMP Study. While *Giardia* may have actually been present more often and at higher concentrations than *Cryptosporidium*, the recovery of *Cryptosporidium* by the analytical method was approximately 10 times lower than *Giardia* in this study.

- The flood samples as a group had the highest geometric mean for *Cryptosporidium*, total/fecal coliforms, *E. coli* , and *C. perfringins* when compared with either the monthly or event sample group results for all organisms. Detection frequency, as percent of positive samples, was higher for all organisms/organism classes in the flood event group than for either the monthly or event sample group.

Correlation Analyses

- The results of the correlation analysis indicate that the data are not well correlated. Only the relationship between fecal coliforms and *E. coli* exhibited a correlation coefficient greater than 80 percent.

- Correlation between the organisms, organism classes, and turbidity are likely to be site specific, and upon further analysis they may also be found to be seasonally specific or episodic.

- Correlation may also be affected by method performance, i.e., the poor precision and accuracy observed in this study for the protozoa may have precluded a quantitative correlation from being determined, should one be present.

- The lack of correlation between the organisms may be due to different ecological characteristics of the species, and surrogates may not be suitable predictions for the occurrence of protozoa.

General

- Experience has demonstrated that both protozoan concentrations and frequency of detection are underestimated by the ICR Protozan Method. An improved analytical method is needed for analysis of *Giardia* and *Cryptosporidium* in raw and finished waters.

- The current ICR Protozoan Method exhibited poor recovery, accuracy, and precision for both protozoans in this and other studies. The method was inadequate based on the high cost and effort required to obtain results, along

with the resulting performance-related qualitative and quantitative limitations placed on the interpretation and use of the data experienced in this study.

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